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ERRATA

Page 538, figure 6, the abscissae of the graph should read .1, .2, .3, .4, .5, .6, .7, .8,
and .9 mm.

Pages 658 and 660, the scale accompanying the figures should read 10 μ .

THE DEVELOPMENT AND DISTRIBUTION OF THE ENDODERMIS AND AN ASSOCIATED OXIDASE SYSTEM IN MONOCOTYLEDONOUS PLANTS¹

D. S. Van Fleet

IN THE course of a morphological and anatomical investigation of the plants in the genus *Smilax*, the author found that a detectable endodermis is generally present in subaerial stems and is absent in aerial stems (fig. 1, 3 and 4). A search through the literature failed to reveal a complete explanation for such an irregularity in the distribution of this tissue in endogenous plants, although Meyer (1881) observed the discontinuous endodermis of *Smilax*, and Schwendener (1883), Müller (1906) and others have recognized the effect of climate and habitat on this dermal layer in monocotyledons. Priestley and his students have described similar irregularities in the endodermis of dicotyledonous plants, and they have given a physiological interpretation to the problem and to the significance of the endodermis.

A preliminary examination of other liliaceous plants brought to light several instances of the same peculiar distribution of the endodermis throughout the stems. A few simple experiments have indicated that it is possible to induce the formation of this tissue in young aerial stems of monocotyledonous plants by introducing substratal factors around the growing stem. It is possible to modify the distribution and maturation of the endodermis by subjecting the growing stems to different types of soil, aeration, light, temperature, etc. Further experiments and observations on the roots and stems of plants in seventeen families of monocotyledons have established a series of basic relationships which may help to clear the way for future work on the development and function of the endodermis.

FACTORS INFLUENCING THE DEVELOPMENT OF THE ENDODERMIS IN ALLIUM ROOTS.—*Materials and methods.*—Since the general influence of environment on endodermal maturation is easier to study in roots, and particularly in roots which develop readily in water culture from bulbs, a series of experiments and observations on the roots of several varieties and species of *Allium* were conducted for the purpose of determining the conditions which influence the development of the endodermis.

The series of lamellae, which in most roots and stems give to the wall of the endodermal cell its characteristic appearance of centripetal development (fig. 2), may be taken as a key to maturational conditions, since these lamellae vary in thickness and in rate at which they are laid down and suberized. By

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The writer wishes to express his appreciation to Dr. Paul Weatherwax for suggesting a study of *Smilax* and for his help and advice in the ensuing investigation. He wishes also to thank Dr. Ralph E. Cleland for his helpful advice and criticism in the preparation of this paper. Acknowledgment is also due Dr. Walter F. Loehwing, Dr. Marion L. Lohman, and Dr. Ira T. Wilson for their suggestions.

sectioning a root at a point where the approximate age is known, it is thus possible to measure the development of the walls of the cells and, knowing their age, to plot their development against various causal and determinative factors. By taking the distance from the lumen of the cell to the middle lamella (fig. 5), it is possible to measure the development of the inner tangential wall in microns with a fair degree of accuracy and ease.

All sections were made free-hand and were taken from a region about one inch from the base of the bulb. By recording the day on which roots made their appearance it was thus possible to know the age of that portion of the root from which the sections were made. The sections were mounted in Scarlet R, Sudan III, Sudan IV, iodine, etc., but, for the most part, Scarlet R was used. No clearing agent was used, since any slight hydrolysis might swell the walls out of their normal configuration. For each causal factor studied, roots from at least three bulbs were used. Allowance was made for diseased or damaged roots, and only the oldest roots were used. Senescence and concurrent injury or disease were the only limiting factors to the duration of the experiments as far as the plants were concerned.

Experiments were designed so as to make possible a study of the rate of centripetal deposition in endodermal walls when influenced by one or more of the following environmental factors: aerated and non-aerated solutions, aerated and non-aerated soils, chemical nutrients, tannic extracts, light, temperature and hydrion concentration. Where water media were used, the bulbs were placed on galvanized wire screens coated with Valspar, and the containers on which the screens rested were either glass battery jars or a similar type of jar or a galvanized tank painted with Valspar. The water was maintained at a constant level by a controlled reservoir supply coming in at a rate slightly greater than the evaporation from the container. A fixed outlet that did not allow the level of the water to drop prevented the base of the roots from being exposed to the air. Where it was desired, the position of the outlets or the screens was changed so that there was a constant exposure of a portion of the roots to the air. A fluctuating water level was obtained by using an automatic siphon, the details of which are too lengthy to present here. Where aeration was needed for a medium, it was accomplished by means of an electric aerator or several devices of a type described by Weatherwax (1915), which bubbled air through the solution. Hydrion concentration was determined colorimetrically, and it was not necessary to modify this factor, since there was for most media, unless otherwise desired, a range of pH 6.2–7.2.

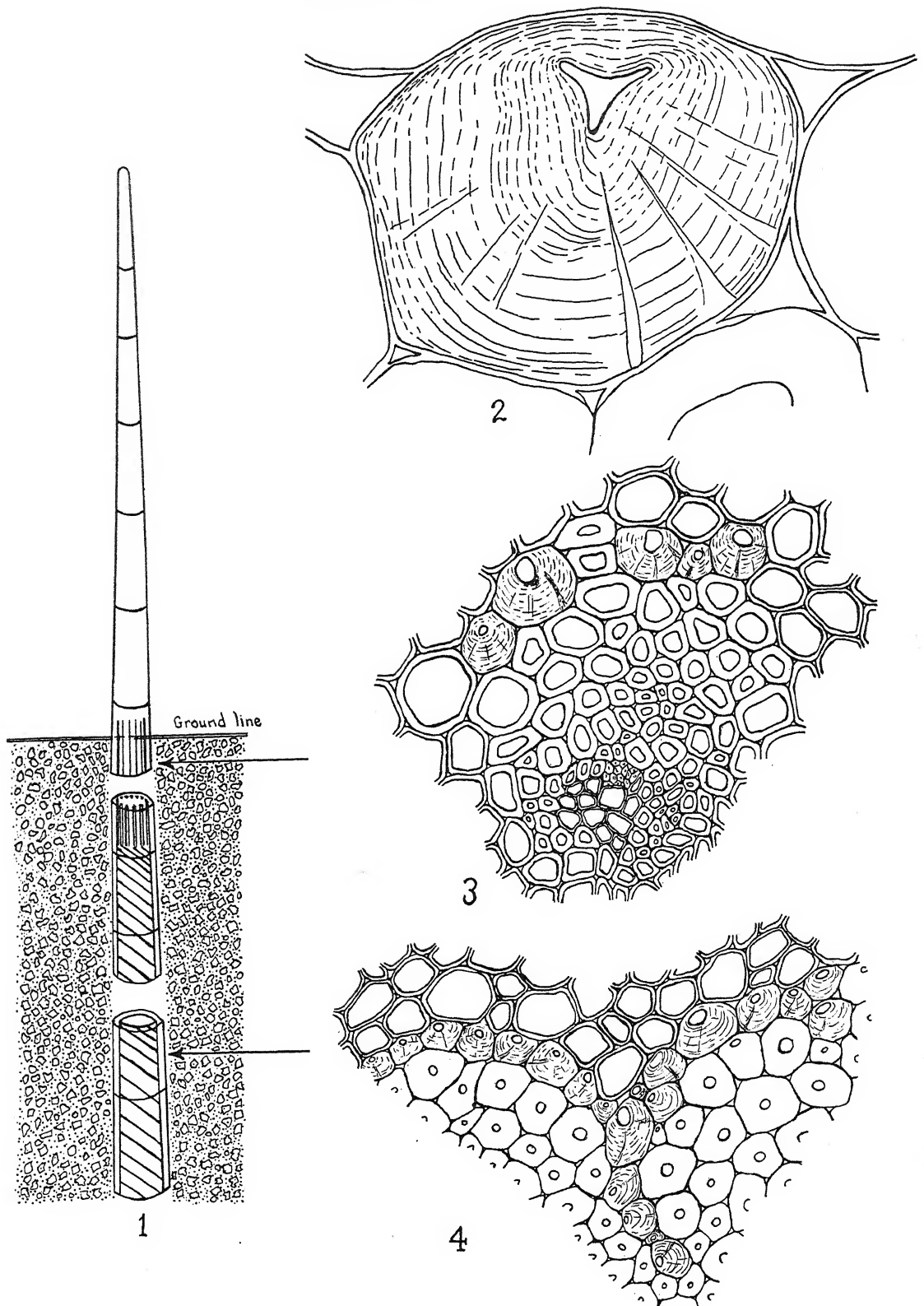


Fig. 1-4. Endodermis of *Smilax* stem.—Fig. 1. Diagram to show cylinder endodermis in internodes below ground with sporadic or bundle endodermis in the region of the ground line and the absence of endodermis in the aerial stem.—

TABLE 1. The influence of the external medium on the centripetal development of endodermal walls in *Allium* roots. Growth period 35 days. Each + sign represents one micron of deposition. Where there was no thickening the reading appears as a dash (—). The symbol —1 is used when the thickening was less than one micron.

Growing conditions	Centripetal deposition in microns		
	<i>A. sativum</i>	<i>A. Ceba</i> (White Globe)	<i>A. Ceba</i> var. <i>solanicum</i>
1. Tap water, fluctuating water level, aerated..	++++++14	+++4	—1
2. Sand, fluctuating water level, not aerated...	++++++14	++2	—
3. Peat, aerotropic roots on surface.....	++++++10	+1	+1
4. Peat, roots below surface.....	+++++6	—1	—1
5. Clay, aerotropic roots on surface.....	++++++10	+1	+1
6. Clay, roots below surface.....	+++++5	—1	—1
7. Tap water, not aerated, light.....	+++++8	—1	—
8. Tap water, not aerated, dark.....	+++++5	—1	—
9. Tap water, aerated, light.....	+++++8	+1	—
10. Tap water, aerated, dark.....	+++++6	+1	—
11. Tannic extract, not aerated.....	++2	—1	—
12. Sandy loam, constant moisture, not aerated.	+++++5	+1	++2
13. Calcium nitrate solution, aerated.....	+++++5	+1	—
14. Potassium nitrate solution, aerated.....	+1	—	—
15. Tap water, not aerated, accumulation CO ₂ ..	+1	—	—
16. Distilled water, not aerated, light.....	+++++5	—1	—
17. Distilled water, aerated, light and dark....	+++++5	—1	—

Each type of *Allium* studied was subjected to a series of individual experiments which imposed a distinct influence or set of influences on the endodermis. The results of these individual experiments are presented in graphic form in tables 1, 2 and 3. The individual experiment is given a number and the conditions of the experiment are listed in the first column, thus making it easy to compare the effect of the various environments on the endodermis of a

given species or variety, and at the same time making possible a comparison between varieties.

The development of the endodermis in roots of *Allium sativum*.—As indicated in table 1, the conditions which serve to give the quickest maturation and suberization of the endodermis result from a constant fluctuation of moisture conditions and aeration. If the roots are alternately bathed with well aerated water and exposed in such a way that they become

TABLE 2. Experiments on *A. Ceba* (Yellow Danver). Nutrient solutions in both aerated and non-aerated water-cultures were used for a growth period of twenty days. Where centripetal development was less than one micron the reading is —1.

Nutrient solution	Centripetal development of endodermis
^a Soft growth solution (high nitrogen) aerated.....	—1 micron
Soft growth solution (high nitrogen), non-aerated.....	—1 micron
^a Hard growth solution (low nitrogen), aerated.....	2 microns
	opposite phloem
	—1 micron
	opposite xylem
Hard growth solution (low nitrogen), non-aerated.....	—1 micron
Solution lacking nitrogen, aerated.....	—1 micron
Solution lacking nitrogen, non-aerated.....	2 microns
	opposite phloem
Complete nutrient solution, aerated.....	—1 micron
Complete nutrient solution, non-aerated.....	—1 micron
Solution lacking calcium, aerated.....	—1 micron
Solution lacking calcium, non-aerated.....	—1 micron
Solution lacking potassium, aerated.....	—1 micron
Solution lacking potassium, non-aerated.....	—1 micron
Solution lacking iron, aerated.....	—1 micron
Solution lacking iron, non-aerated.....	—1 micron

^a Withrow and Biebel (1937).

Fig. 2. Typical endodermal cell with centripetally deposited lamellae.—Fig. 3. Bundle endodermis from region near ground line as indicated by arrow.—Fig. 4. Cylinder endodermis from below ground.

TABLE 3. Observations on various species of *Allium* grown in loam under conditions of fluctuating water supply.

Species	Growing conditions	Growth period	Development of endodermis opposite phloem
<i>A. canadense</i>	Sandy loam	80 days	8 microns
<i>A. Cepa</i> var. <i>Bermuda</i>	Tap water non-aerated	30 days	—1 micron
<i>A. tricoccum</i>	Humus	80 days	8 microns
<i>A. flavum</i> major	Sandy loam	160 days	6 microns
<i>A. album</i>	Sandy loam	160 days	8 microns
<i>A. speciosum</i>	Sandy loam	160 days	4 microns
<i>A. oreophilum</i>	Sandy loam	160 days	5 microns
<i>A. tanguticum</i>	Sandy loam	160 days	7 microns
<i>A. odorum</i>	Sandy loam	160 days	4 microns
<i>A. ammophilum</i>	Sandy loam	160 days	8 microns
<i>A. pyrenaeicum</i>	Sandy loam	160 days	3 microns
<i>A. sphaerocephalum</i>	Sandy loam	160 days	6 microns

dry but not desiccated, an endodermis will develop (table 1, experiment 1) which is quite in contrast with that attained by growing under conditions of poor aeration (table 1, experiment 7). This treatment, however, will not induce as elaborate a development of endodermis as will subjection to a fluctuating water level in sand (compare experiments 2 and 10 in table 1).

Perhaps the most conclusive evidence derived from the work on *A. sativum* is that obtained from aerotropic roots (fig. 5, and experiment 3 and 5 in table 1). The portion of the root above the ground develops a greater centripetal thickening in the endodermis than that below ground. It may be inferred from the evidence that the amount of gas which goes into the cortex, as well as incipient desiccation, acts as a stimulus to the centripetal thickening of the walls.

A fluctuating water and gaseous supply evidently far overshadows any other set of external environmental conditions, such as salts, light, etc., in its effect on endodermal maturation in *A. sativum* roots. The extreme reverse of these conditions would be where the roots are subjected to a decreased or detrimental gaseous supply as, for example, in a carbonic acid gas medium (experiment 15, table 1). The un-aerated tannic extract medium (experiment 11, table 1) quite evidently has an inhibitory effect which will be explained later, and which will assume considerable significance in the light of evidence which will be presented on an oxidase system which the author has found to be associated with endodermal wall formation.

The development of the endodermis in roots of Allium Cepa (White Globe).—Like *Allium sativum*, *Allium Cepa*, of the White Globe variety, also shows the greatest centripetal development of endodermal walls when subjected to a fluctuating water level (experiment 1, table 1), whereas constant exposures of the roots to air and to aerated and non-aerated water media do not produce any remarkable differences in their effect on the elaboration of the endodermis. The behavior of endodermal cells in a calcium nitrate solution (experiment 13, table 1) is one of hurried

maturation, and it diverges from the usual slow ex-centric development. This was also true in *Allium sativum*.

Similar experiments were conducted on other varieties of *A. Cepa* (Red Wethersfield, White Seed, and Yellow Danver) with almost identical results. The endodermis of White Seed Onions responds to a fluctuating water level just as does the Mature White Onion (White Globe). The young bulbs and old bulbs both produce roots whose endodermal cells mature at about the same rate in the same media. It should be pointed out at this time that the removal of the outer scales from a bulb results in a more pronounced development of endodermal walls of the root. This was also true in White Globe bulbs which had been sectioned transversely into two halves, the injured bulb producing roots with a more pronounced endodermal development than is found in roots from normal bulbs. The mature bulbs of the Red Wethersfield type respond readily to exposure to air and to a fluctuating water level. The seed onions of this variety are slow in the maturation of their endodermal cells in ordinary aerated tap water, as are also the seed onions of the White Globe type. When roots of the Red Wethersfield are exposed to air, the maturational rate is increased. Again aeration and exposure of the roots are conducive to endodermal maturation in Yellow Danver onions. It is of interest to note that the Casparian strip develops in experiment 15 (carbon dioxide) as it always does in this variety, regardless of the type of aeration. This would lead one to believe that the Casparian strip represents an anaerobic phase in the development of the endodermis, or that there is a cortical oxygen supply from the bulbs. This was also found to be true in *A. sativum*.

A study of the various nutrients as they influence the development of the endodermis in *A. Cepa* (Yellow Danver) in both aerated and non-aerated solutions (table 2) indicates that solutions low in nitrogen are conducive to heavier deposition of ligneous and suberous materials. Solutions high in nitrogen do not bring about centripetal deposition, but solutions high in calcium do give a more marked deposition.

In general the concentration of any one salt does not have as great an influence on the development of the endodermis as does the extent of aeration. Experiments being conducted at present indicate that a fluctuation in concentration has about the same influence as a fluctuation in water and gas supply.

The development of the endodermis in roots of Allium Cepa var. solanicum.—As in the aerotropic roots of *A. sativum*, the portions of roots of *A. Cepa* var. *solanicum* grown above the substratum show a more rapid centripetal thickening of the endodermal walls than those portions grown below (experiments 3 and 5, table 1). A slightly fluctuating water supply in a loam medium (experiment 12, table 1) has a more pronounced influence on the rate of suberogenic substances than does the fluctuation of the water level in water culture. There is no difference between aerated and non-aerated tap water or distilled water so far as the early development of the endodermis is concerned (experiments 7, 8 and 12, table 1), and apparently light or the absence of light does not incite any difference in the development of the endodermis in the same medium (experiments 7, 8, 9 and 10). Aerated and non-aerated tap water, whether in the light or dark, has the same, or about the same, influence as aerated or non-aerated distilled water in the light or the dark.

The influence of tannic extracts on the development of the endodermis in the roots of three types of onions.—Tannin apparently acts as a retarding factor in the unilateral differentiation of endodermal walls. Its action is to take out of solution the available dissolved oxygen. This is evidenced by the "browning" of the solution as a result of oxidation. It is further possible that it may act as an anti-oxidant; this is a point which is further suggested by observations on oxidase activity. In the three types of *Allium* subjected to this medium it is quite evident that tannins do not alter the trend of response. The difference in development between *A. sativum* and *A. Cepa* var. *solanicum* in tannic extract is typical of the difference between these two types in several media, and it is further true that *A. Cepa* var. *solanicum* shows the least centripetal development of all forms studied regardless of the medium of growth. From these preliminary observations and conclusions, it becomes evident that the apparently fundamental difference between the various types of onions in respect to response of endodermal tissues is perhaps in part due to the presence of inhibitive tannins. These tannins are present most prominently in the scale leaves of *A. Cepa* var. *solanicum*, and it is this species which shows the maximum effect of some inhibitive or retarding force. The tannins may occasionally be found in the bases of the roots, and they have in a few cases been found to occupy a place in the developing tangential walls of the endodermal cells. *A. sativum*, of the variety used, is notably free of tannins in both bulb and root. The White Globe, Yellow Danver and Red Wethersfield varieties apparently occupy an intermediate position both in re-

sponse to media and in quantity of tannins present in their tissues.

The difference between surface and substratal portions of the same roots (aerotropic) in two types of onions.—As was stated earlier in connection with aerotropic roots of *Allium sativum* (see fig. 1) the aerial portions of the roots show at least twice as great a centripetal thickening as do the walls of even older cells from the subaerial portions. The response of the three types of onions is again indicative of an inherent pattern which is difficult if not impossible to break or alter. Again the three types remain distinct in their expression, but *A. Cepa* var. *solanicum* shows the least tendency to respond. As subsequent data may show, there may be a passage of inhibitory substances from a portion of an organ in one medium to a portion in another medium. This is particularly true where injury to the bulb results in a greater development of the endodermis in roots on the injured bulb.

A comparison of three types of onions in their response to a fluctuating water level.—This medium represents the optimum condition for centripetal expression in the endodermis of *A. sativum* and *A. Cepa* (White Onions); however, *A. Cepa* var. *solanicum* is characterized by a greater centripetal thickening of endodermal walls in a sand medium with a fluctuating water level, or in a fluctuating water level in sawdust. The author believes that *A. Cepa* var. *solanicum* develops a more definite cuticular barrier than the other two forms. Sand and sawdust media for some reason apparently retard the formation of a functional cuticle. In a portion of a root freely exposed to moist or dry air and under light conditions favorable to cuticular deposition, there is a possibility of an interrelationship between cuticle formation and the development of the endodermis. This relationship is more strikingly shown in stems than in roots.

Conclusions based on experimental evidence obtained by varying the environmental growing conditions for Allium roots.—Obviously there is an inherent physiological pattern that may be modified; and, even for any one variety, the pattern remains rather fixed. There is no one factor more significant than another in determining the maturational rate. Some general statements can be made, however, as to the effects of certain sets of factors or basic environmental relationships that influence the rate of maturation. These relationships, as summarized from the tables, may be stated briefly as conclusions.

Neither constant dryness nor constant moisture, especially under conditions of poor aeration, are conducive to endodermal maturation. A fluctuation of dry-moist conditions, accompanied by constant aeration, is apparently most conducive to deposition. Various nutrients apparently do not greatly modify the rate of deposition. The absence of dissolved oxygen in a solution or very moist medium tends to retard greatly the deposition of suberic substances. The accumulation of carbonic acid gas in the medium, or its artificial introduction, allows the formation of the

Casparian strip alone and retards suberic deposition. In some cases tannic substances are almost as effective as carbonic acid in retarding suberic deposition. The section on oxidases will make clear the exact relationship of tannins to endodermal maturation. The presence or absence of light apparently does not have any effect except as it probably modifies the amount of gaseous and dissolved oxygen in the medium and tissues of the plant. A condition of incipient desiccation and plasmolysis resulting from external environmental factors is recognized as a possible explanation for centripetal development of endodermal walls.

Discussion.—The author, in attempting to clarify the formational factors in the development of the endodermis, has gone back to Schwendener's (1883) premise that, "Hieraus erklären sich zugleich die Beziehungen zu Klima und Standort . . . die Wurzeln der Felsen- und Steppenpflanzen verstärkt Scheiden besitzen. . . . Wo dagegen der Standort constant weich und feucht bleibt . . . da sind die Scheiden der Wurzeln ohne mechanische Verstärkungen. . . ." This statement has been taken as a basis upon which to start this investigation.

The necessity of oxygen for the formation of cuticular and suberic substances, found in dermal barriers, was first noted by Kny (1889). He demonstrated that the exclusion of oxygen from the cut surface of a potato will prevent the formation of wound periderm. Priestley and Woffenden (1922), in studying the causal factors in cork formation, have confirmed the results of Kny and other workers on the necessity of oxygen for suberization. Priestley and North (1922) concluded that the mature endodermal walls are made up of condensation products of suberogenic or fatty acids not found in cork. Later Priestley and Rhodes (1926) made a macrochemical study of the endodermis and found suberin-like materials, similar to those in potato cork, present in endodermal cells in the tertiary or mature stage. They pointed out that it is probable that suberin lamellae arise from fatty substances as a result of oxidation and drying and stated further, "It has been demonstrated that the occurrence of these complexes depends on the air supply." Priestley (1921) had earlier stated that suberin may be regarded as "an aggregate of variously modified forms (condensation products or anhydrides)." Thus it is that earlier workers came to associate oxygen or desiccation with suberic deposition; and, more recently, Bloch (1935, 1937), in studying wound healing in *Tradescantia* and in roots of *Phoenix reclinata*, has shown that suberization and formation of wound gum are dependent upon an adequate oxygen and sap supply.

It should not be supposed that increased oxygen supply in the medium will bring a response in suberin-depositing tissues alone. There is a danger in assuming that suberin is the only substance deposited in endodermal and wound tissues. Loehwing (1934) has pointed out that there is an increase in size of organs and nutrient absorption and an increase in

wall thickening of all cells in roots of continuously aerated plants. Bryant (1934) also has found the thickness of the walls of all cells of barley roots to be twice as great in aerated as in non-aerated solutions. Oxygen and adequate aeration are necessary for all types of deposition, and this must be taken into account in any study of suberic and related depositions. Hence, it is quite possible that dissolved oxygen has some effect on the ultimate sources of suberin rather than on the actual mechanism of deposition.

Priestley (1924), and Priestley and Lee (1924) have presented evidence that the calcium in the substratum is responsible for the immobility and deposition of suberin in roots so that there is less deposition in stems. They further believe that potassium acts to bring the soluble condition and free mobility of these same fatty acids up into the stems. Hence suberization and cuticularization are thought to be increased in stems but not in roots by an increase in potassium or a decrease in calcium in the soil.

The author has found suberization to be prominent in the endodermis of the mesocotyl of corn plants grown in solutions of calcium nitrate. Plants of the same age grown in a potassium nitrate solution show only a weak Casparian deposit, and the cells are in some cases about 30 per cent larger in a radial direction and in total volume. The endodermal cells opposite the xylem in normal roots are often radially enlarged and are more rounded than those opposite the phloem; this condition is, I believe, indicative of a difference in osmotic relationships between tissues, and the nitrate solutions apparently disturb these normal relationships.

Millner (1934) has made an ecological anatomical study of *Silene vulgaris* in which he finds that the conditions of the soil have a bearing on suberization. He finds that clay-grown plants show the least periderm and only a slight suberization, whereas sand-grown plants show a copious suberization, and other soils are intermediate in their effect. This conclusion is, in general, a valid one for suberization and endodermal maturation in *Allium* according to the results I have obtained, and it restates very clearly the observations and inferences originally made by Schwendener (1883).

*AN OXIDASE SYSTEM ASSOCIATED WITH THE ENDO-
DERMIS.*—The exact mechanism of suberization in dermal layers has never been fully understood. Drabble and Nierenstein (1906) were perhaps the first to suggest and demonstrate that suberin is formed from the condensation of tannins and fats. Priestley and his students also believe suberization to be a condensation reaction and that the oxidation or condensation of unsaturated fatty acids takes place wherever suberin lamellae are being formed. However, oxygen and the necessary suberic substances may often be present, and suberin lamellae will still not form unless, for example, the tissue is wounded or becomes dry. Desiccation alone is not a primary causal factor in suberic condensation, since the relative humidity must be fairly high for most

suberization, and it is also apparent that a definite excess or minimum of oxygen is not essential. This would suggest that there is perhaps a more complete mechanism than has heretofore been proposed.

Tunmann has stated (1913, p. 427) that tannins are changed to phlobaphenes by oxidases. Priestley and North (1922) found tannins of the phlobaphene group associated with the endodermis of *Pteridium aquilinum*, and the author has found phlobaphenes in the monocot endodermis. Grüss (1910), Tunmann (1913), and others have pointed out the existence of oxidases in wounded tissues, although there is nothing in the literature on their causal relation to cicatrization and suberization. Samish (1935) has found oxidases in apricot fruits located around, but not in, stelar tissue. Sanglet (1928) has found oxidases localized for the most part in the bundle sheaths of *Ilex* leaves.

The author has made a few preliminary observations and has found an oxidase system associated with endodermal tissues and mestome or bundle sheath tissue in many monocotyledonous plants. It is not intended that the material presented here be taken as proof of a causal relationship between oxidases and endodermal maturation; it is my belief, however, and it will be plain in the discussion which follows, that the presence of an oxidase system in endodermal tissues may be a causal factor in endodermal and related suberic depositions. As a result of this study certain other observations have been made on the effect of the mature endodermis on oxidase distribution, and it is believed they indicate a further relationship between the function of the endodermis and the ultimate development of other tissues around it.

The endodermal oxidase system.—Solutions of various oxidase indicators were used to determine colorimetrically the presence and location of oxidases in, and associated with, the developing and mature endodermis. Many of the reagents and methods used by Onslow (1920, 1921, 1931) and Joyet-Lavergne (1935, 1937) and outlined by Boswell and Whiting (1940) were employed to determine the presence of both oxidases and reductases. Solutions of aloin, aesculin, vanillin, alpha-Naphthol, paraphenylenediamine, "Nadi" reagent, meta-phenylenediamine, benzidine, diphenylamine, diphenylbenzidine, guaiacol, and various oxidation-reduction indicators were used for the detection of oxidases in living sections of roots and stems. For much of the work guaiacol and "Nadi" reagent were used in the direct reaction for the detection of organic peroxides and peroxidases. Hydrogen peroxide was not used very often with guaiacol in the indirect guaiacol reaction, since in most cases the direct reaction indicated the presence of organic peroxides located in the walls and intercellular spaces of endodermal cells, and peroxidases were almost always present. Sodium cyanide, hydrogen sulphide, boiling water or alcohol either inactivate, remove, or precipitate the organic peroxides, but they are apparently not removed as readily as the peroxidases. Cold alcohol does not remove the oxi-

dase system. Oxidized guaiacol, benzidine, and "Nadi" reagent are only weakly absorbed in endodermal cells or walls. Hence the localization of colorimetric reactions is not entirely an adsorption phenomenon.

There are oxidases in the developing Casparian strips of *Allium* roots (fig. 6) and also in the intercellular spaces. Similarly the underground stems of

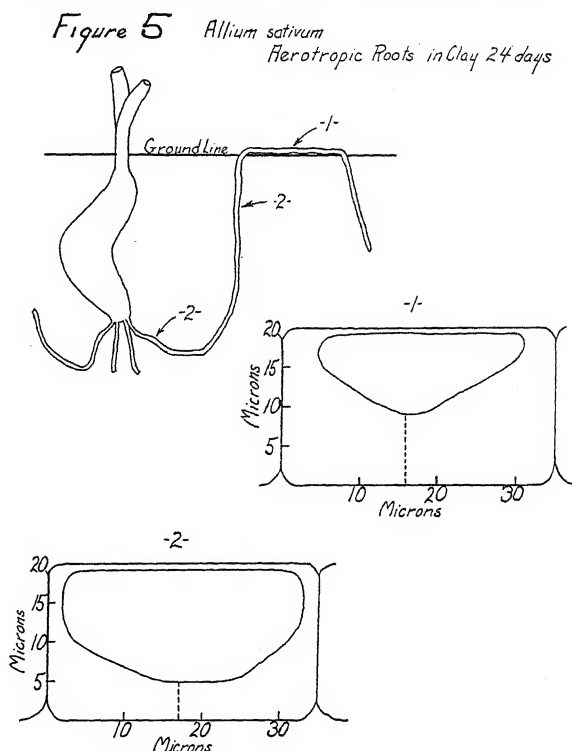


Fig. 5. Arabic numerals 1 and 2 indicate two distinct portions in the same root, the above ground aerotropic portion (1) and the below ground portion (2). The condition of the endodermis in these two regions is indicated by the diagrams numbered 1 and 2 to indicate the portion of the root from which they were taken.

Smilax glauca (fig. 8) and *Smilax ecirrhata* have an oxidase system located in the developing Casparian strip, the plasma membrane, and the intercellular spaces. The inner tangential walls of the endodermal cells of *Smilax ecirrhata* show an oxidase system associated with suberic deposition. These walls are the first to show a suberin lamella. The centripetally developing endodermal cells of *Maianthemum* stems (fig. 7) have oxidases in the portions of the wall which are youngest, next to the lumen, before the deposition of tannic substances which apparently inhibit the oxidases. The quantity of tannin present in younger lamellae is not sufficient to mask the colorimetric determination. *Maianthemum* roots like those of *Allium* have oxidases in the developing Casparian strip (fig. 9) and also in the inner tangential walls, which at the same time or a little later have suberic lamellae deposited on them.

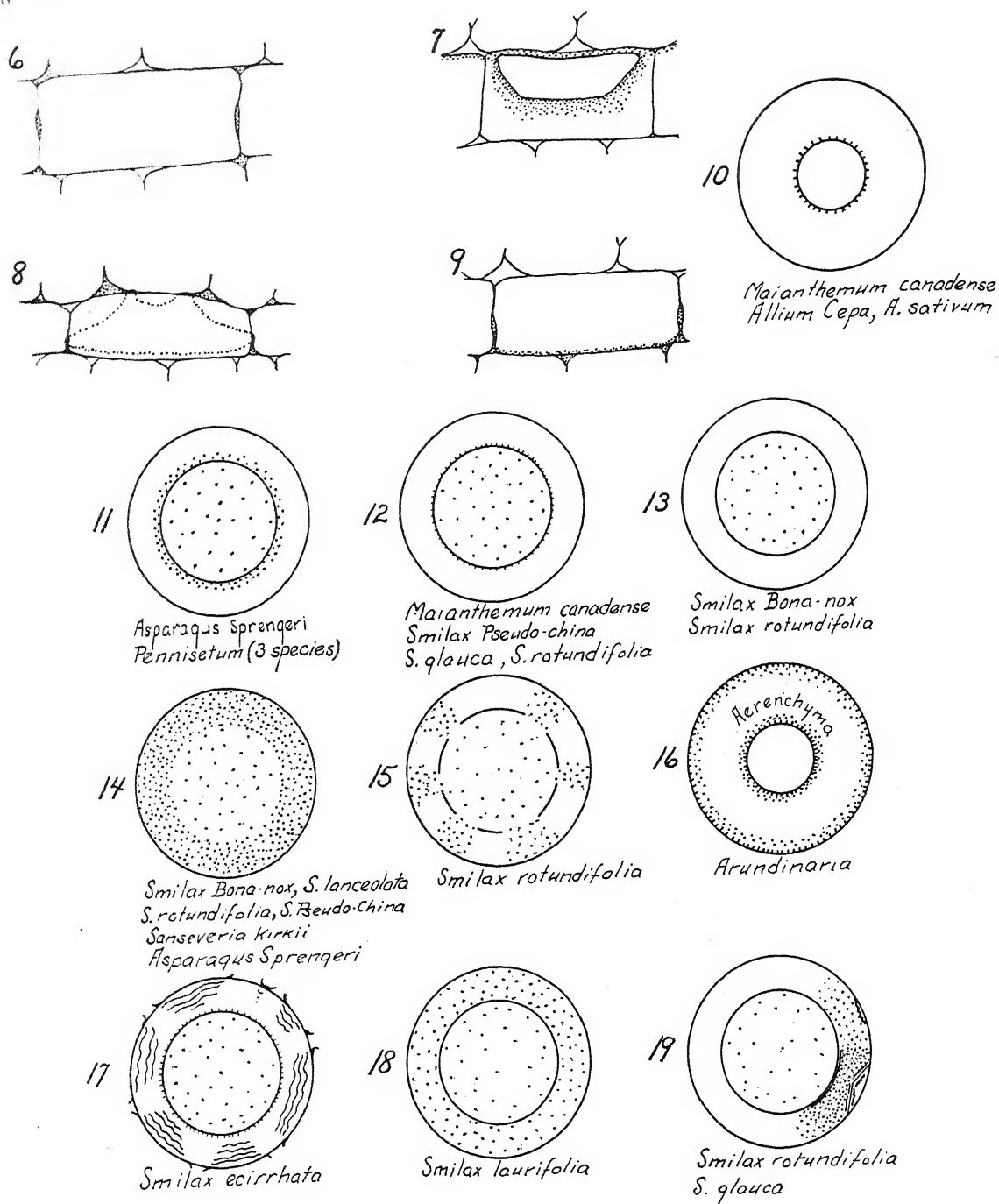


Fig. 6-19. Stippling indicates extent and relative quantity of oxidase activity.—Fig. 6-9. Oxidases associated with development of endodermal deposits.—Fig. 6. Endodermal cell of *Allium* root showing Casparian strip.—Fig. 7. Centripetal deposition in endodermis of *Maianthemum* stem with greatest oxidase activity in younger lamellae next to lumen.—Fig. 8. Endodermal cell with Casparian strip deposits near four intercellular spaces in stem of *Smilax glauca*.—Fig. 9. Endodermal cell from *Maianthemum* root.—Fig. 10-19. Diagrams of stem and root transverse sections to show distribution of oxidases and endodermis.—Fig. 10. Diagram of root to show complete cylinder endodermis and oxidases more prevalent inside endodermis than in cortex.—Fig. 11. Stem, complete endodermis and more or less confined oxidases.—Fig. 12. Stem, cylinder endodermis and confined oxidases.—Fig. 13. Same.—Fig. 14. Absence of endodermis in aerial stem and oxidases not confined to stele.—Fig. 15. Oxidases in cortex where gaps occur in endodermis in stem in region of ground line.—Fig. 16. Root, oxidases associated with hypodermis and endodermis.—Fig. 17. Decorticated stem and confined oxidase system.—Fig. 18. Poorly developed endodermis in subaerial stem and oxidase system

Oxidase activity is greater in those endodermal cells opposite the phloem, and this, of course, is related to the fact that endodermal cells show the greatest centripetal thickening in this region. The cells opposite the xylem have Casparian strips and associated oxidase activity in these same radial walls and strips; however, oxidases are not as prevalent in the tangential walls of endodermal cells opposite the xylem, and these walls often remain unsuberized throughout the life of the tissue. This was observed in the roots of *Aloe*, *Dracaena*, *Smilax*, *Allium* and numerous other forms. Likewise the suberic centripetal development of the mestome sheath is most prominent opposite the phloem portion of the bundle, and it is in this region that oxidase precursors are apparently most prevalent. Colorimetric determinations show oxidases in the phloem and across the intervening sclericycle between phloem and endodermis with the most intense reaction in the endodermis and the phloem. We have, therefore, concluded from these and other observations that the oxidase system has its origin in phloem, in part, and that it becomes functional along with suberic precursors at the endodermal barrier.

The absence of the endodermis in aerial stems of monocotyledonous plants may be due to the absence of a complete oxidase system at the locus of the endodermis. Preliminary tests indicate that catechol tannins are more prevalent in young underground stems than they are in young aerial stems. The oxygenase which catalyzes the production of a peroxide from these catechols may be inactivated by light. At least one or both of these two essentials to organic peroxide formation are not present in detectable quantities at the peripheral edge of the sclericycle in aerial stems but are found in small amounts throughout the cortex. This may have some bearing on the absence of the endodermis in aerial stems. At the same time there is the relationship to cuticle formation of the endodermis. I have found that the cuticle is from 10 per cent to 75 per cent thicker above the surface of the soil than it is below, and Priestley and Lee (1924) have found this to be true of dicotyledonous plants. Coupled with this I have observed that in aerial stems the oxidases apparently do not halt at the histogenic endodermal zone, but move on out to hypodermis and epidermis. Hence, it is conceivable that the cuticle is functional at an early stage and prevents the inward disturbance of normal oxygen gradients so that an excess of oxygen or a condition of incipient desiccation and plasmolysis is prevented. The oxidase system would thus not meet an oxygen supply adequate for the formation of organic peroxides in quantities sufficient to release active oxygen to aid in suberic deposition. It is interesting to note the prevalence in many young *Smilax* stems of anthocyanins in the peripheral regions of the cortex at the ground-line. This suggests an oxidase system and an oxygen-holding system which might act to prevent the inward movement of oxygen at a time when

the cuticle is not fully developed. The endodermis does not form in this region at all. There are some further observations listed below which may clarify this very interesting and complex relationship.

In *Pennisetum longistylum*, *P. Ruppelianum*, and *P. japonicum* the direct reaction with guaiacol shows the greatest oxidase activity in underground stems and rhizomes, in the zone of the endodermis. The oxidases are not confined to this region in the aerial stem but are found throughout the cortex. *Asparagus Sprengeri* (fig. 11) also has a confined oxidase system in portions of the stem below the surface. In some cases the oxidases are located only in the outer tangential walls of endodermal cells. In aerial stems the oxidase system is not confined but is spread throughout the cortex.

In *Maianthemum canadense* (fig. 12) oxidases are present within the endodermal walls in the rhizome of young developing tissues, but in November, when the plant is dormant and after tannins have accumulated, the oxidases are apparently inhibited or their precursors are not present. Similarly in underground stems of *Smilax glauca* (fig. 12) the oxidases are in the intercellular spaces and the outer tangential walls, and are masked by the tannins in the inner tangential walls, although it is true that even at younger stages, before tannins can be detected (by ammonia vapors, oxidizing agents), the oxidases are largely confined to the outer tangential walls. Both aerial and underground portions of etiolated *Smilax glauca* stems show the same confinement of the oxidase system as indicated by the direct reaction with guaiacol. *Smilax rotundifolia* (fig. 12) has its oxidase system confined where there are conditions which lead to endodermal formation, but in aerial stems (fig. 14) the system is spread throughout the cortex. This is also true of the distribution of oxidases in the stems of *Smilax Bona-nox*.

Decorticating stems of *Smilax ecirrhata* (fig. 17) have a highly active oxidase system which is most intense within the tangential walls of the endodermis. The inner tangential walls and the radial walls of endodermal cells in decorticated plants of *Smilax ecirrhata* and *Smilax herbacea* show particularly well the presence of oxidases.

The endodermis of *Smilax laurifolia* (fig. 18) is not always well defined in underground portions of the stem, and it is often not unilaterally thickened, whereas most *Smilax* endodermal cells are very thick-walled (fig. 2). The endodermis in this species does not always form a complete cylinder; and, even when it does, it is only possible to identify it by using hydrolytic reagents on the sections. The oxidase system is not confined but is present throughout the cortex.

Natural or artificial wounding of the cortex of *Smilax rotundifolia* and *S. glauca* (fig. 19) results in an oxidase system which is largely confined to the zone of the wound and is more intense just outside and in the endodermis than it is in the endodermis of

not confined.—Fig. 19. Wounded stem with greatest oxidase activity in region of wound and greater deposition in endodermis in this region.

normal unwounded tissue. The endodermis at this point is much more strongly developed than in other portions of the same stem.

Portions of mature underground stems of *Smilax Bona-nox* (fig. 14) which lack an endodermis show a natural oxidation and browning of the entire cross-section, and oxidase indicators show the same results. Water extracts of cortical tissue, from stems which lack an endodermis, give a faint blue color by the direct guaiacol reaction and a deeper blue color on the addition of hydrogen peroxide. Cortical extracts from mature stems with an endodermis (fig. 13) do not give either the direct or the indirect reaction with guaiacol.

Dormant stems of *Smilax rotundifolia* (fig. 14), either aerial or substratal stems, which lack an endodermis, will show natural browning of the entire fresh section within about five minutes. Likewise oxidase tests by the direct reaction show a complete oxidase system throughout the cortex. Where the endodermis is discontinuous (fig. 15), the oxidase system in the cortex is confined to those portions of the cortex where there are gaps in the endodermis. It must be pointed out here that the cuticle is sometimes fifteen microns thick in substratal stems of *Smilax rotundifolia* which lack an endodermis, whereas it is less than eight microns thick in stems which possess an endodermis. A thick functional cuticle may prevent the inward sweep of excessive amounts of gas so that the oxidase system perhaps does not become complete at the endodermal histogen. This histogen is really just the boundary between two distinct physiologic systems, the cortex and the stele or the cortex and the sclericycle, and it is, I believe, logical to suppose that effusa from one system may become halted or deposited at the boundary of another. Horizontal underground stems of *Smilax rotundifolia*, which run along in a layer of clay covered by humus, often lack an endodermis but have a thick cuticle. If one of these stems is cut off, or if the terminal bud is killed, the branches which develop from other buds back of the tip will possess an endodermis regardless of whether they continue as prostrate stems or whether they grow vertically upward through the humus layer to become aerial stems. The cuticle of these stems is appreciably thinner, and the oxidase system is confined, whereas in the main stem it was complete in its distribution. Back of the cut surface of the parent stem there is an accumulation of organic peroxides and tannins. There is undoubtedly a causal connection between the accumulation of these substances and the development of the endodermis. The author is conducting an experiment which may give some additional and more conclusive evidence.

Certain *Arundinaria* roots (fig. 16) are peculiar in that they have a centripetally thickened hypodermis whose inner tangential walls are thinnest, so that they are the mirror images of the endodermal cells whose outer tangential walls are thinnest. Between these two dermal layers there is a large aerenchymal cavity or series of cavities. The oxidase system is

confined to the hypodermal and endodermal layers and to the intercellular spaces immediately adjoining these dermal barriers.

Discussion of endodermal oxidase system.—Oxidases in the endodermis have not hitherto been recorded, but their presence in related tissues, the bundle sheaths, have been pointed out by Keeble and Armstrong (1912), Sanglet (1928), and Samisch (1935). The significance of oxidases to the maturation and function of any one tissue has not been made clear, although there are many papers which deal with the distribution of oxidases and the difference in the oxidation and oxidation potential in the organs of the same plant.

Raciborski (1898a and 1898b) was one of the first to point out oxidases in the phloem. As I have pointed out, endodermal development and oxidase activity are both more pronounced opposite phloem than opposite xylem. Oxidases may be traced by indicators from the phloem out to the endodermis, and that oxidative activity is greater in this region is evidenced by natural browning of the tissue. It is possible that the oxidase system moves out from the phloem in all directions, but the conditions are most favorable for its expression in the endodermis, sclericycle, or cortex. If oxidases move in toward the center of the stele, they are inhibited or are in some manner incomplete. All of the examinations that I have made lead me to the conclusion that the oxidase system in the endodermis has its immediate origin in the phloem, and the greatest centripetal development is opposite the phloem. Scott (1928) and others have observed that the Casparian strip does not appear before phloem elements are differentiated.

The intense oxidation of aromatic substances to give a definite browning of the tissues outside the phloem is accomplished only where a definite endodermal barrier has been formed. The endodermis tends to hold the constituents of the oxidase system so that they are accumulated and destroyed or held in abeyance. The inhibition of the system is dependent upon the presence of dissolved oxygen and the release of active oxygen from the organic peroxides of the system. The accumulation of oxidized aromatics, or in other words the accumulation of tannins, tends to inhibit the oxidase system. As a result, at the endodermis there is a constant fluctuation between the oxidation and deposition of tannic and suberic substances, and the inhibition of the oxidase system. It follows, therefore, that oxidation is not complete, and this is indeed true as demonstrated by the unsaturated nature of the suberic substances in the cellulose matrix (as indicated by iodine, osmic and Sudan stains).

If the fatty acid precursors to suberization were completely oxidized there would be no deposition. Deposition may include an oxidase system and accumulated tannic substances acting against each other so as to bring about incomplete oxidation and centripetal deposition. This fluctuating system, responsible for fatty acid deposition, may be the thing that is influenced by environmental factors, and there

may be no direct environmental influence on fatty acid deposition itself.

The observations presented here on the confinement of the oxidases to a given region in substratal organs and the associated presence of an endodermis may not be too clearly established as a causal relationship in the maturation of the endodermis, but there are some other observations which are indicative of conditions which have their influence on maturation in terms of an enzymatic system. For example, it is possible that calcium nitrate solutions do not influence suberization directly, as postulated by Priestley and his students, but rather that such solutions affect oxidase activity. Oxidase activity is higher in onion bulbs of the anthocyanic type than in those of the albino variety as pointed out by Wheldale (1916) in reviewing Katic's work. Thus in the bulbs of *Allium Cepa* var. *solanicum* the oxidase activity is higher than in the bulbs of *Allium sativum* and *Allium Cepa* of the White Globe variety as evidenced by the presence of anthocyanins in the former and their absence in the latter. Anthocyanin is an oxygen acceptor, and it may be argued that the high oxidizing properties of anthocyanic tissue might affect the formation of organic peroxides which find their way to the roots or the amount of dissolved oxygen which enters the roots from the bulbs.

Summary of endodermal oxidase system.—An oxidase system is associated with endodermal maturation, and it may be causally related to the location and ultimate formation and function of the mature endodermis. The following statements may be made as to the possible relationship of oxidases and related factors to the formation of the endodermis.

When the cuticular barrier is poorly developed, the increase in the gaseous content and incipient desiccation in the cortex brings about the confinement of the oxidase system to the locus of the ultimate endodermis. The oxidase system is not confined when the cuticle is well developed. The oxidase system helps to bring about, by means of active oxygen, the oxidation of fatty acids and certain aromatic substances. The oxidized aromatics counteract oxidase activity and fatty acid oxidation, so as to bring about a state of fluctuation in oxidation, and incomplete oxidation results. The centripetal development of the endodermal walls and unsaturated nature of suberin are evidences of this fluctuation in oxidation and deposition. The oxidase system originates in the phloem. At least the organic peroxide precursors come from the phloem, and it is probable that their activators also come from the phloem. A part of the oxidase system may be blocked by the mature endodermis. The first suberin layer may be a "shock" layer which influences and helps to bring about later deposition. Oxidase activity is increased by incipient desiccation and by wounding.

THE DISTRIBUTION OF THE ENDODERMIS IN MONOCOTYLEDONOUS PLANTS.—There has not been a tabulation of the distribution of the endodermis in monocotyledonous plants since Müller's (1906) excellent survey of the literature and additions to it through

his own observations. Müller divided the plants which he and others had studied into those with stems lacking a cylinder endodermis and those with a cylinder endodermis. The extent of the endodermis was not carefully considered in subaerial and aerial stems. Early plant histologists recognized the endodermis only in those cases where there was a very conspicuous Casparian strip or a mature condition of obvious unilateral deposition. The mestome sheath or bundle sheath was considered as a distinct tissue allied to the endodermis but not a true endodermis (Schwendener, 1888). By hydrolyzing cell walls with zonite, hydrochloric acid and zinc chloride, etc., many cells, which by their appearance belong either to the cortex or sclericycle, are shown to be actually endodermal cells since the washing away of younger lamellae reveals Casparian strips. This is also true of many, but not all, mestome or bundle sheath cells of *Cyperus*, *Carex*, *Zebrina*, *Tradescantia*, and others.

Unilateral deposition is not always a good criterion for the identification of the endodermis because cells other than the true endodermis become centripetally thickened (Russow's " ϕ Zellen"; see Schwendener, 1888, or Müller, 1906). The author has found only a few instances of the absence of the Casparian strip where unilateral deposition will take place or has taken place. Hence, the Casparian strip is often an excellent guide to what constitutes an endodermal tissue both in appearance and point of origin. Unilateral deposition usually takes place in cells which have earlier developed Casparian strips. These two distinct types of deposition are almost without exception confined to the same tissue in monocotyledonous plants. The masking of the Casparian strips by later deposits has led all the early investigators to overlook some endodermal tissues and to regard them as another type of tissue. This was true of the interpretation of the mestome sheath by Schwendener (1890), Wille (1915), and others.

Physiologic position thus becomes more significant than any anatomical position with reference to stele or cortex. The critical physiologic barrier between cortical extremes on the one hand and phloem and xylem conditions on the other will give an endodermis which varies greatly in its distribution in a single plant. For example, in *Tradescantia aureo-striata*, there is a cylinder endodermis below the surface and at the same time a bundle sheath type of endodermis surrounding some internal as well as peripheral bundles. The aerial prostrate stems of this plant lack a cylinder endodermis at times so that only a bundle sheath is present, and it is not recognizable as an endodermis unless hydrolyzing agents are employed. The tissue does not serve as a guide to the anatomy of a plant so much as it does to developmental and functional conditions within tissues.

A tabulation of the distribution of the endodermis in monocotyledonous plants, as determined by the author, is presented in appendix 1. Each plant in this list was examined with a view to determining the distribution of the endodermis in aerial and sub-

aerial organs, or its distribution in aerial and subterranean portions of the same organ. As an example of the way in which each plant has been studied, the case of *Smilax* may be presented (fig. 1, 3 and 4). The cylinder endodermis (fig. 4) completely surrounds all the bundles except in the region of the surface of the ground where the endodermis becomes broken and is present only opposite the phloem in each bundle (fig. 3). Above the surface of the ground there is not a detectable endodermis.

THE STARCH SHEATH.—It is difficult to detect the presence of oxidases in the starch sheath of *Dioscorea* and *Smilax* stems. In other parts of *Smilax* plants where oxidases are present in the endodermis, the starch granules are absent. It is conceivable that oxidases and starches would not appear at the same time in the same tissue. A disappearance of starch from wounded tissue has been observed by Brieger (1924), and he found that the starch was especially strongly removed from cells having an oxidase reaction. Biedermann and Jernakoff (1924) believe that oxidases are capable of doing diastatic work. The disappearance of starch and the appearance of the endodermis having a Casparian strip is thus conceivably due to the presence of an oxidase system. Thus the nature and distribution of the oxidase system may influence endodermal expression and be a determining factor in the presence or absence of starch in the endodermis.

A definite suberic pellicle surrounds groupings of starch granules in the starch sheath of some aerial stems of *Smilax glauca* and *S. herbacea*. This starch is resistant to diastase and ptalin solutions longer than potato starch placed in the sections and longer than other naturally occurring starches in the stele. Sections heated in hydrochloric acid-zinc chloride and treated with iodine show a breaking down of the starches of the stele as indicated by the erythrodxetrin color, but starches of the starch sheath remain blue until they are heated for a longer period of time. It is thus possible that starches are retained in the starch sheath because those cells can suberize or otherwise render the starch impervious to enzymatic, hydrolytic and oxidizing agents.

THE CASPIAN STRIP.—It has long been recognized that the Casparian strip is a deposit of lignic or suberic materials on or in the radial walls of endodermal cells. The origin of the strip has not been fully explained, although it is generally agreed that fatty and related substances pass out of the stele and diffuse between the radial walls of the cells of the endodermis. These substances, it is believed, become deposited at some exact locus in the radial walls to give a strip of uniform width.

Some observations lead me to conclude that the Casparian strip has its origin as a deposit in the inner intercellular space of the endodermal cells. With an increase in the size of the cells in a radial direction the strip comes to occupy a position farther from the intercellular cavity. In the roots of *Smilax glauca* there first appears a deposit in the inner intercellular space formed by two radial walls. Later this deposit

may be found a few microns from the intercellular space, and finally it becomes permanently located about five microns from the edge of the cavity. Thus with an increase in size in a radial direction the Casparian strip comes to occupy a more central position between the radial walls. In one instance, in roots of *S. glauca*, I found another intercellular deposit, smaller than the first, developing after the first strip became permanently located five microns from its point of origin. In roots of *Iris* there is occasionally a double Casparian strip. The first one to appear has its origin near or actually in the inner intercellular cavity, and the other develops a little later at the outer intercellular cavity. The double Casparian strip occurs opposite the xylem, but not opposite the phloem. The osmotic relations in the region of the xylem, I believe, bring about the radial enlargement of endodermal cells so that they measure from two to five microns larger, in a radial direction, than do the endodermal cells opposite the phloem. As a result of the addition of new material and expansion of the walls, this, then, results in a relocation of Casparian deposits and sometimes the formation of new ones. In the roots of *Aloe* there is often an increased radial growth opposite the xylem so that the deposit is farther from the intercellular cavity in endodermal cells in this region.

After the strip becomes permanently fixed in one place, the radial walls around it, or the entire radial wall, may take on lignosuberic substances which become deposited and obscure the strip, so that it loses its identity as an intercellular deposit. It is this mature condition which probably led some of the early anatomists to the conclusion that each cell laid down a deposit on the inside of its radial walls. It is indeed highly probable that there is some internal deposition in the formation of the strip.

The irregularity in the distribution of the endodermis in monocotyledonous plants and the different types and rates of its maturation are indicative of the full significance of what may be thought of as *position effect* in tissue differentiation. The endodermis does not always occupy the same histogenic position. Casparian strips may develop in cells other than the histogenic endodermal layer, and they develop in terms of the surrounding tissues. For example, in stems of *Smilax glauca* the Casparian strips may be found running obliquely and quite irregularly around the radial walls so that the strip is actually cleft and divided in a few places. These irregular strips may occupy more than a single layer of cells, particularly in those portions of stems close to or at the surface of the ground. It is also commonly true in *Smilax* stems that the fascicular endodermal layer is not the same histogenic layer of cells that is present between the bundles as interfascicular endodermis. Thus by physiologic position a layer of cells comes to have Casparian strips and unilateral deposition.

SUMMARY

Experiments indicate that, in the roots of *Allium*, the rate and ultimate extent of the development of

the endodermis are governed, in part at least, by certain sets of environmental factors. An alternation of dry and moist conditions, accompanied by constant aeration, is apparently most conducive to the centripetal development and deposition of the walls of endodermal cells. Incipient desiccation and plasmolysis is recognized as a possible explanation for centripetal development. Several factors, such as light, temperature, salts, and organic substances, have a lesser influence on the maturation of this tissue and particularly as they may affect an oxidase system associated with the endodermis in its development.

The centripetal development of endodermal cell walls, the unsaturated nature of suberin, and the irregular distribution of the endodermis are evidences of a fluctuation in oxidation and deposition. This fluctuation is brought about by cortical, stelar and environmental factors which influence the activity, distribution, and nature of an oxidase system associated with the endodermis. Thus, a functional cuticular barrier prevents the movement of gases across the cortex; hence the oxidase and depositional precursors do not become confined to the endodermal layer but are spread throughout the cortex. If, on the other hand, the cuticle is not functional and does allow the diffusion of gases and the formation of a higher oxygen gradient across the cortex, then the oxidase system becomes confined and complete at the endodermal barrier and brings about characteristic deposition in this dermal layer.

The Casparian strip has its origin, at least in some cases, as an extracellular deposit first formed in the intercellular space, later occupying its characteristic position between the radial walls due to the radial growth of the cells.

The endodermal starch sheath is low in oxidases, which apparently accounts for the retention of starch and the absence of centripetal deposits characteristic of the endodermis. The retention of starch in the endodermis, to form a starch sheath, seems to be due, in some instances at least, to the deposition over each grain of a thin but clearly detectable pellicle of suberic substance which does not permit access of hydrolyzing enzymes to the starch. Hydrolytic agents are slower in bringing about the hydrolysis of this starch than of other starches in the plant.

Using a new definition of endodermal tissue, the distribution of endodermis has been determined in 17 families and over 90 genera and 100 species of endogenous plants.

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APPENDIX

The distribution of the endodermis in stems of monocotyledonous plants.—The cylinder endodermis, modified bundle sheath and related tissues may be classified on the basis of their distribution in stems, modified stems, and leaves of monocotyledonous plants. The following categories have been es-

tablished as a result of personal observation in most cases (indicated by *), but some of the plants listed have been taken from the literature in cases where it was possible to authenticate the statements of the authors or to reinvestigate at least the genus. Authorities accredited with the original observations are listed by name and date of publication after the name of the plant whose endodermis they studied.

ENDODERMAL TISSUES PRESENT IN HORIZONTAL AND ERECT UNDERGROUND STEMS.—ALISMACEAE: **Alisma Plantago-aquatica*, *Alisma Plantago* (Sauvageau, 1891), **Sagittaria sagittifolia* (Guillaud, 1878). AMARYLLIDACEAE: **Doryanthes Palmeri*. ARACEAE: **Acorus Calamus* (de Bary, 1877, and Guillaud, 1878), **Acorus gramineus* (de Bary, 1877), **Symplocarpus foetidus*. BROMELIACEAE: **Ananas sativus*. CANNACEAE: **Canna indica*. CYPERACEAE: **Carex picta*, **Carex vulpinoidea*, **Carex vulpina*, **Carex arenaria*, *C. leporina*, *C. paniculata*, and *C. hirta* (Schwendener, 1890); **Scirpus atrovirens*, *S. cernuus*. IRIDACEAE: **Belamcanda chinensis*, *Iris florentina* (Guillaud, 1878), *Iris versicolor*. LILIACEAE: **Allium tricoccum* (prophyllar bulb connective), **Aloe chinensis* (Mangin, 1882), **Aspidistra elatior* (Chrysler, 1903), **Camassia esculenta*, **Convallaria majalis* (Mangin, 1882, and Müller, 1906), **Sansevieria Kirkii*, **Sansevieria Zeylanica* var. *aurantia*, **Veratrum Woodii*, **Yucca gloriosa* (seedling). NAIADACEAE: *Zostera marina*, and *Z. nana* (Sauvageau, 1891). ORCHIDACEAE: **Aplectrum hyemale*. PALMACEAE: **Chamaedorea elatior* (Guillaud, 1878), **Phoenix dactylifera* (seedling). TYPHACEAE: **Typha angustifolia* (Müller, 1906).

ENDODERMAL TISSUES PRESENT IN ERECT UNDERGROUND STEMS, CULMS OR SCAPES UP TO THE GROUND LINE WHERE IT DROPS OUT, OR WHERE IT DROPS OUT AS A CYLINDER ENDODERMIS TO BECOME A BUNDLE SHEATH.—AMARYLLIDACEAE: **Agave americana* var. *marginata*. BROMELIACEAE: **Ananas sativus*. COMMELINACEAE: **Tradescantia Crassula*, **T. aureo-striata*, **T. reflexa*, **Zebrina pendula*. CYPERACEAE: **Carex trichocarpa*, **Scirpus atrovirens* and others of this genus. LILIACEAE: **Allium sativum* (scape), **Allium canadensis*, **Asparagus officinalis*, **A. Sprengeri*, **Convallaria majalis* (Müller, 1906), **Hosta japonica*, **Maianthemum canadense*, **Medeola virginiana*, **Oakesia sessifolia*, **Ruscus aculeatus*, **Sansevieria carnea* (lower side of rhizome, Müller, 1906), **Smilacina racemosa*, **S. stellata* (not always), **Smilax Bona-nox* (not always), **S. ecirrhata*, **S. pseudo-china*, **S. laurifolia*, **S. lanceolata* (not always), **S. glauca*, **S. rotundifolia* (not always), **S. herbacea*, **S. hispida*, **Smilax China* (Meyer, 1881). SPARGANIACEAE: **Sparganium simplex*. ZINGIBERACEAE: **Hedychium gardnerianum*.

ENDODERMAL TISSUES PRESENT IN AERIAL PORTIONS OF ERECT, OR PROSTRATE STEMS, IN DECUMBENT AERIAL STEMS, AND IN CULMS AND SCAPES AS A CYLINDER ENDODERMIS.—ARACEAE: **Scindapsus pictus* (Müller, 1906). COMMELINACEAE: **Commelina virginica* (erect and prostrate), **Tradescantia fluminensis* (both cylinder endodermis and bundle sheath, Priestley and Scott, 1925). CYPERACEAE: **Carex picta*, **C. vulpinoidea* (stem), **Cyperus strigosus*. IRIDACEAE: **Iris versicolor*. LILIACEAE: **Asparagus asparagoides* var. *myrtifolium*, **Chlorophytum elatum*, **Medeola asparagoides* (Müller, 1906), **Smilax ecirrhata*. ORCHIDACEAE: **Epipactis pubescens*, **Vanilla planifolia* (Müller, 1906).

ENDODERMAL TISSUE PRESENT IN LEAVES BELOW THE SURFACE, AND PORTIONS OF SUBAERIAL LEAVES (EITHER AS A CYLINDER ENDODERMIS OR AS A BUNDLE SHEATH).—AMA-

RYLLIDACEAE: **Agave americana* var. *marginata*.
BROMELIACEAE: **Ananas sativus*. CANNACEAE:
**Canna indica*. IRIDACEAE: **Iris versicolor*. LILIA-
CEAE: **Allium tricoccum*, **Aloe latifolia*, **Aspidistra*
clatior, **Hosta japonica*, **Smilax* spp. (scale leaves).

ENDODERMAL TISSUES ABSENT IN SUBAERIAL STEMS WHICH
DO NOT GROW UP TO THE GROUND LINE.—ARACEAE: *Arum*
italicum, *A. maculatum* (van Tieghem, 1886), **Arum crin-*
itum, **Colocasia esculenta*. AMARYLLIDACEAE:
**Agave Parryi*, **Hymenocallis occidentalis*, **Narcissus*
Pseudo-Narcissus, **Polygonatum tuberosa*. DIOSCOREA-
CEAE: **Dioscorea Batatas*, **D. villosa*. LILIACEAE:
**Allium* spp., **Aloe latifolia*, **A. variegata*, **Camassia*
esculenta, **Gasteria verrucosa*, **Kniphofia Tuckii*, **Mus-*
cari comosum, **Nothoscordum fragrans*, **Polygonatum*
biflorum, **P. commutatum*; **Polygonatum latifolium*, **P.*
multiflorum, **P. officinale* (Chrysler, 1903); **Polygonatum*
vulgare (Guillaud, 1878); **Trillium sessile* (Chrysler,
1903); **T. recurvatum*, **T. declinatum*, **Tulipa* spp.,
**Yucca glauca*, **Y. filamentosa*. ORCHIDACEAE: **Co-*
rallorhiza maculata, **Orchis spectabilis*.

ENDODERMAL TISSUES ABSENT IN BOTH AERIAL AND SUB-
AERIAL PORTIONS OF THE STEM, SCAPE, OR CULM, AND ALSO
LEAVES.—ALISMACEAE: **Alisma Plantago-aquatica*
(scape), **Sagittaria latifolia* (scape). AMARYLLIDA-
CEAE: **Doryanthes Palmeri* (leaf), **Narcissus Pseudo-*
Narcissus (scape), **Polygonatum tuberosa*. ARACEAE:
**Aglauonema simplex* (stem), **Amorphophallus giganteus*
(scape and petiole), **Arisaema dracontium*, **A. triphyl-*
lum (scape and petiole), **Peltandra virginica* (Müller,
1906) (stem and scape), **Philodendron hastatum*; **P. Rud-*
geum (van Tieghem, 1886); **P. cordatum* (stem),
**Symplocarpus foetidus* (scape). COMMELINACEAE:
**Rhoeo discolor* (stem). DIOSCOREACEAE: **Dioscorea*
Batatas, **D. villosa* (stem). LILIACEAE: **Aloe arbo-*
rescens (stem), **A. latifolia* (leaf), **Camassia esculenta*
(scape), **Dracaena indivisa* (stem and leaf), **Erythro-*

nium americanum (Chrysler, 1903) (scape), **Lilium re-*
gale (leaf), **Muscari comosum* (leaf and scape), **Orni-*
thogalum umbellatum, **Polygonatum biflorum*, **P. com-*
mutatum (stem); **Trillium sessile* (Chrysler, 1903)
(stem); **T. recurvatum*, **T. declinatum* (stem), **Tulipa*
spp. ORCHIDACEAE: **Aplectrum hyemale* (scape),
**Corallorhiza maculata* (scape), **Habenaria flava*
(scape), **Liparis liliifolia* (scape and leaf), **Orchis spec-*
tabilis, **Pogonia ophioglossoides* (scape), **Spiranthes cer-*
nua (stem and scape). PALMACEAE: **Livistona mauri-*
tana (de Bary, 1877), **Phoenix dactylifera* (leaf).

ENDODERMAL TISSUES PRESENT ON BOTH SIDES OF THE
SCLERICYCLE.—*Clintonia umbellulata* (Chrysler, 1903),
Luzula campestris (Guillaud, 1878), **Medeola virginiana*
(stem), **Scirpus validus* (rhizome), **Uvularia perfolia-*
tum (stem).

BUNDLE SHEATH OR MESTOME SHEATH TYPE OF ENDODER-
MAL TISSUE IN AERIAL PORTIONS OF LEAF AND STEM.—**Carex*
picta, **C. trichocarpa*, **C. vulpinoides* (leaf and stem),
**Cyperus strigosus*, **Eriocaulon septangulare* (around bun-
dles in cortex but not around those inside the stele) (Solo-
mon, 1931), **Oplismenus Burmannii* (leaf), **Pennisetum*
longistylum, **P. Ruppelianum*, **P. japonicum*, **Scirpus*
atrovirens, **S. cernuus*, **S. validus* (stem and leaf), **S.*
cyperinus, **Tradescantia fluminensis*, **T. aureo-striata*,
**Zebrina pendula*. CYPERACEAE: Present in most spec-
ies (Plowman, 1906; Wille, 1915). GRAMINEAE: The
following classification is by Schwendener (1883). Leaf
bundles with a mestome sheath: *Festuceae*, *Bambuseae*,
Aenaceae, *Hordeaceae*, *Phalarideae*, *Alopecuroideae*,
Agrostideae, *Stipaceae*, *Chlorideae*, *Arundineae*, *Oryzeae*,
Panicaceae. Leaf bundles without a mestome sheath: *Andro-*
pogoneae, *Maydeae*, *Panicaceae*. I have examined several
grasses having a mestome sheath made up of cells with
Casparian strips as shown by hydrolysis. Corn and various
members of the *Andropogoneae* and *Panicaceae* do not have
cells of the bundle sheath with Casparian strips.

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STUDIES OF SOUTH AFRICAN PHAEOPHYCEAE. I. ECKLONIA MAXIMA, LAMINARIA PALLIDA, MACROCYSTIS PYRIFERA¹

George F. Papenfuss

THE PRESENT paper is the first of a short series dealing with the development in culture of certain South African Phaeophyceae. The forms treated in this account are representative of three of the five families of the order Laminariales, viz., Alariaceae (*Ecklonia maxima*), Laminariaceae (*Laminaria pallida*), and Lessoniaceae (*Macrocystis pyrifera*). The results obtained agree with those of other investigators of this order. For the pertinent literature reference should be made to the papers of Kylin (1933), Hygen (1934), and Kanda (1936). The papers of McKay (1933) and Hollenberg (1939) contain valu-

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able information on the process of fertilization, and the former author gives a good account of the chromosomal cycle in the life history of *Pterigophora californica*.

METHODS.—The culture methods followed were those used by Kylin (1933) and others. Fertile parts of the plants were kept out of water for several hours or over night in covered glass jars. Pieces of the material were then placed in glass dishes containing filtered sea water and slides arranged on end along the sides of the dishes. After a period ranging from about thirty minutes to several hours, depending upon the number of zoöspores that had become attached to the slides, the latter were removed, all spores wiped from the one surface and the edges, and filtered sea water poured over the slides in order to wash off as many diatoms as possible. The slides were then placed on end in glass tumblers containing the nutrient solution,² the surface containing the spores facing the side of the tumbler. The tumblers were covered with glass dishes and placed in front of a southeast window in an unheated basement room, precaution being

² The culture fluid used was the one employed by Schreiber (1930). It consists of: NaNO_3 , 0.1 gr.; Na_2HPO_4 , 0.02 gr.; distilled water, 50.0 cc.; sea water, 1,000.0 cc.

taken that they were at no time exposed to direct sunlight. The nutrient solution was not renewed except when the cultures were kept for many months. In certain instances distilled water only was added at intervals to replace the water lost by evaporation. Desired stages of the sporelings were fixed in position on the slides with Flemming's weak solution for a period of five to ten minutes. The fixative was removed by rinsing the slides in sea water. A few drops of glycerine were then added to the slides and the lat-

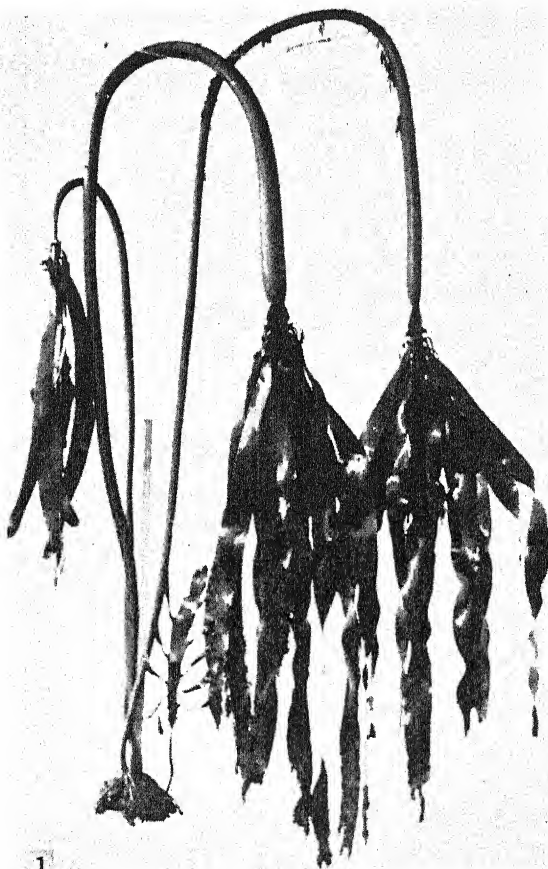


Fig. 1. *Ecklonia maxima*, habit of plant, about 1/20 nat. size.

ter kept in covered boxes for several weeks to allow the glycerine to become concentrated. Cover-glasses were then placed on the slides and the latter sealed and stored for future study. In such preparations the sporelings retain their normal form and most of their color; no staining is necessary unless it is desired to study the cytology of the plantlets.

ECKLONIA MAXIMA (OSBECK) PAPENF.—*General remarks*.—This plant has been known for a long time as *Ecklonia buccinalis*, but it was recently pointed out by Papenfuss (1940) that the specific name *maxima* has priority.

The known South African distribution of *E. maxima* extends from Port Nolloth on the west coast

(Bright, 1938) to a place, Papenkuilsfontein, about six miles west of Cape Agulhas on the south coast, where the writer found it growing in abundance in 1937. This distribution is not continuous, however, for the species is absent, or is represented only by occasional stunted individuals, along the northern and the greater part of the western shores of False Bay. The absence or scarcity of *E. maxima* in this region is owing no doubt to the high sea temperatures that prevail there during the summer months.³ Isaac (1938), who has studied the temperature conditions of the South African coastal waters, states (p. 25): "It is safe to assume that, where more or less normal sized *Ecklonia buccinalis* grows, the mean annual temperature does not exceed about 14.6°C." The high temperature conditions that eliminate *E. maxima* from the flora of the northern and western parts of False Bay are apparently less marked or do not obtain along the central and southern parts of the eastern shore of this bay and in consequence the species reappears at certain stations along this coast, starting at the mouth of the Steenbras River, and extends eastward beyond False Bay as far as the locality near Cape Agulhas referred to above.⁴

In addition to South Africa, *E. maxima* has been reported from the following parts of the world: St. Paul Island in the southern ocean (Grunow, 1867), Tristan da Cunha (Kolben, 1719), Falkland Islands (Bory, 1826), Chile (Postels and Ruprecht, 1840).

The presence of *E. maxima* at St. Paul is not improbable, as this island has a number of other marine algae in common with South Africa. The occurrence of the species at any of the other localities is extremely doubtful, however. The record for Tristan da Cunha is based on Kolben's (1719, p. 298) statement that the plant was observed floating in the vicinity of the island during his voyage to South Africa in 1705. It is not clear from Kolben's account, however, that he actually was near Tristan da Cunha. According to Dr. E. Baardseth, algologist of the recent Norwegian expedition to Tristan da Cunha, the species does not occur at the island (personal communication). The record for Falkland Islands is based on the plant that Bory (1826, p. 594) described as *Laminaria flabellum*, a species that he (Bory, 1828, p. 99) later considered as a variety of *E. maxima*. Certain subsequent authors have also regarded *L. flabellum* Bory as being identical with *E. maxima*, but Hooker (1845, p. 160) and others are doubtful of the correctness of this interpretation. The Chilean record of Postels and Ruprecht (1840, p. 3) is based on specimens that were secured from the drift. Judging from their figure (plate 11), it seems very doubtful that the plant is *E. maxima*. The figure shows a specimen in which the pinnae are split

³ For a discussion of the factors that cause these high temperatures in this part of False Bay, see Isaac (1937a, pp. 658-659).

⁴ De Toni (1889, p. 790; 1895, p. 358) refers to a specimen of *E. maxima* in Herb. Kew that came from Algoa Bay. If this record is correct the specimen must have been obtained from the drift, as *E. maxima* does not grow in this region.

longitudinally to the base into several segments, a feature that I do not recall having seen in *E. maxima*.

In the juvenile state, the thallus of *E. maxima* consists of a simple, somewhat elongated, blade borne on a stipe. In slightly older stages pinnae develop along both margins of the blade, as will be seen from the youngest specimen in figure 1. The pinnae are initiated in the meristematic region at the base of the

The sori form broad elongated patches on both surfaces of the pinnae. Fertile plants were obtained during all months of the year but March, for which month the writer has no field data on this species.

Development of gametophyte.—The zoöspores like those of most other Laminariales lack an eyespot and exhibit no phototactic response. In this connection, it is of interest to note that in the laminarian

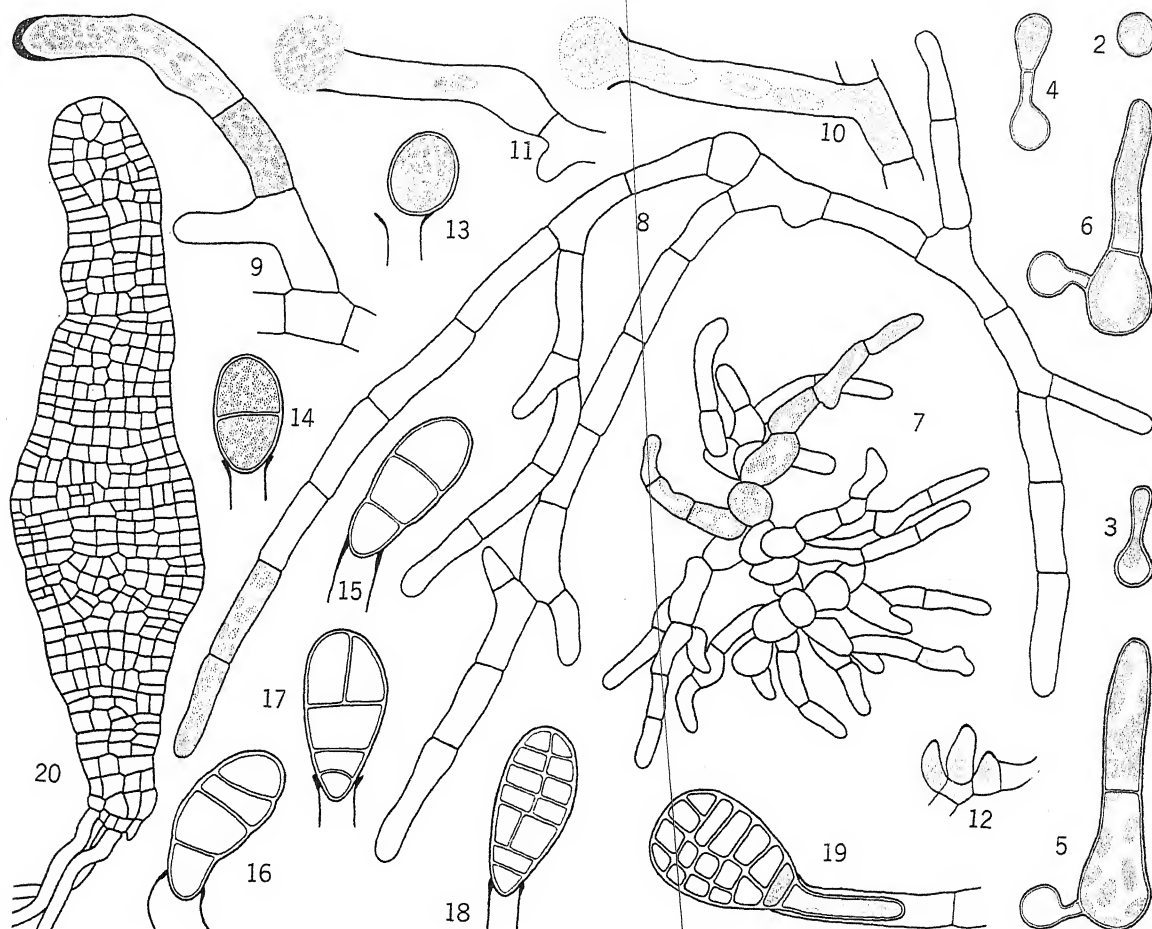


Fig. 2-20. *Ecklonia maxima*.—All figures are camera lucida drawings of plants grown in nutrient solution.—Fig. 2. Rounded zoöspore, $\times 990$.—Fig. 3. Gametophyte, 1 day old, $\times 990$.—Fig. 4. Same, 3 days old, $\times 990$.—Fig. 5. Same, \varnothing , 15 days old, $\times 990$.—Fig. 6. Same, δ , 15 days old, $\times 990$.—Fig. 7. Same, δ , 25 days old, $\times 650$.—Fig. 8. Same, \varnothing , 25 days old, $\times 440$.—Fig. 9. Oögonium with cytoplasm in an early stage of contraction, $\times 440$.—Fig. 10. Extrusion of egg, $\times 440$.—Fig. 11. Extruded egg, $\times 440$.—Fig. 12. Cluster of antheridia, one antheridium empty, $\times 990$.—Fig. 13. Zygote, $\times 440$.—Fig. 14-20. Stages in development of sporophyte. Fig. 14-19, $\times 440$. Fig. 20, $\times 290$.

primary blade and are progressively shifted upward by elongation of the latter. Later the distal portion of the primary blade is worn away and the pinnae become aggregated in a dense cluster immediately above the transition region (fig. 1), the older pinnae continually wearing away and new ones being initiated. In older plants, the stipe becomes hollow and its terminal portion becomes inflated, forming a large float. Mature plants may attain a length of seven meters or more from base of stipe to tips of longest pinnae.

Eisenia arborea the zoöspores show no reaction to light even though they possess an eyespot (Hollenberg, 1939).

After a period of motility ranging from a few minutes to several hours, the zoöspores come to rest, assume a rounded form, and secrete a wall about themselves (fig. 2). Germination sets in shortly afterward and in cultures that are twenty-four hours old many germlings may have well-formed germ tubes, and the single chromatophore may have divided once (fig. 3). As development continues, the chromato-

phores and most of the cytoplasm migrate into the terminal portion of the germ tube; and in cultures that are three days old this portion of the tube usually has been delimited by a cross wall from the spore case (fig. 4). At this time the chromatophores usually have undergone further division. During the succeeding seven to ten days, growth is more or less limited to enlargement, few additional cells being formed. In cultures that are fifteen days old, it is possible, however, to distinguish between female

tension which serves as the apical portion of the oögonium (fig. 10). The oögonia are readily recognizable by their dense content and by the fact that the cytoplasm pulls away from the wall. When mature the egg is extruded through a rupture that arises at the apex of the oögonium (fig. 10). A small amount of cytoplasm frequently remains in the oögonium. The extruded egg forms a spherical naked protoplast at the mouth of the oögonium (fig. 11), where it usually remains in position. The chromatophores

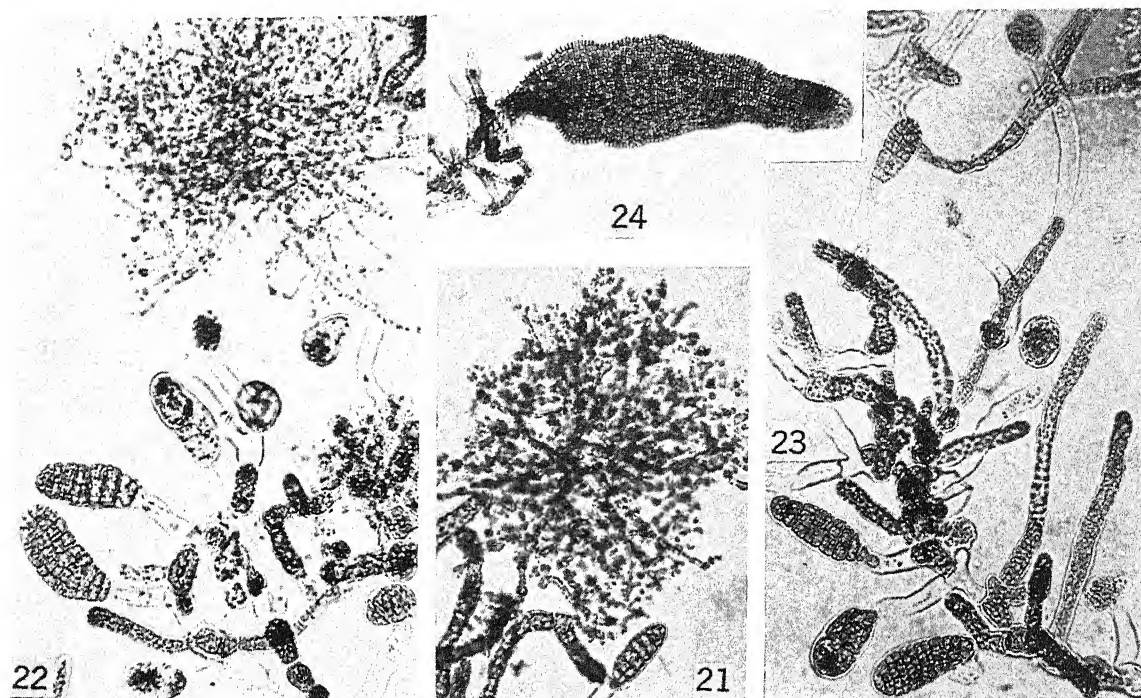


Fig. 21-24. *Ecklonia maxima*.—Photomicrographs of plants grown in nutrient solution.—Fig. 21. Male gametophyte, with portion of female at the lower end, $\times 200$.—Fig. 22. Male and female gametophytes, and several young sporophytes, $\times 200$.—Fig. 23. Female gametophyte with young sporophytes, $\times 200$.—Fig. 24. Young sporophyte, $\times 85$.

(fig. 5) and male (fig. 6) gametophytes, the female plantlets being of a greater diameter than the male. Not infrequently male gametophytes of this age may have formed the first branch and consist of a total of six cells. Subsequent growth continues at a comparatively rapid rate and in cultures that are twenty-five days old the male and female plantlets have assumed the form shown in figures 7 and 8, respectively. In male gametophytes of this age, the cells, especially the terminal ones, generally contain fewer and larger chromatophores than do those of the female plantlets. At this stage the first signs of the differentiation of reproductive organs, especially of the oögonia, may be observed; and in cultures that are thirty-five days old young sporophytes may already be present, although reproductive organs and sporophytes are usually not formed in abundance until the gametophytes are forty to fifty days old.

The oögonia (fig. 9) develop from terminal or from intercalary cells. When an intercalary cell is transformed into an oögonium it forms a lateral ex-

of the egg retain their identity and can be recognized during the entire maturation period of the egg (fig. 9-11).

Male gametophytes (fig. 21, 22) differ from female ones in being slenderer and much more profusely branched. The antheridia (fig. 12) are formed in clusters at the tips of the branches or as outgrowths from intercalary cells. Young antheridia may be distinguished from vegetative cells by their denser cytoplasmic content. As the antheridium matures, the cytoplasm withdraws from the wall and appears as a homogeneous brownish matrix. Each antheridium apparently forms but one spermatozoid.

Development of sporophyte.—Fertilization of the egg was not observed but the first noticeable change which presumably follows this process is the formation of a wall about the zygote (fig. 13). The zygote then elongates and ultimately divides transversely (fig. 14). The next few walls are also in a transverse plane (fig. 15, 16). Longitudinal walls then set in, starting at the terminal end (fig. 17, 18), and

as a result growth takes place in two planes, giving rise to a somewhat elongated monostromatic sporophyte (fig. 23). The plantlet shown in figure 19 has formed its first rhizoid. At times a second rhizoid may have been formed at this stage but usually it is formed considerably later. The rhizoids are always nonseptate and are usually unbranched; branched rhizoids were but rarely observed. The chromatophores present in a young rhizoid later degenerate.

The young sporophytes become distromatic at a comparatively early stage. The first cells to divide periclinally are those in the basal region, the part

prevail in these regions during the summer months (cf. Isaac, 1937b, p. 146).

In addition to South Africa, *L. pallida* has been reported from St. Paul Island in the southern ocean (Grunow, 1867, p. 51); the Canary Islands, the Atlantic coast of Morocco, the coasts of Portugal and northwestern Spain, and the southwestern part of the Mediterranean (cf. Dangeard, 1936, p. 101; Feldmann, 1934, p. 13). The plants from St. Paul probably are of this species, but those from the northeastern Atlantic and the western Mediterranean have been shown by French algologists to be of a distinct

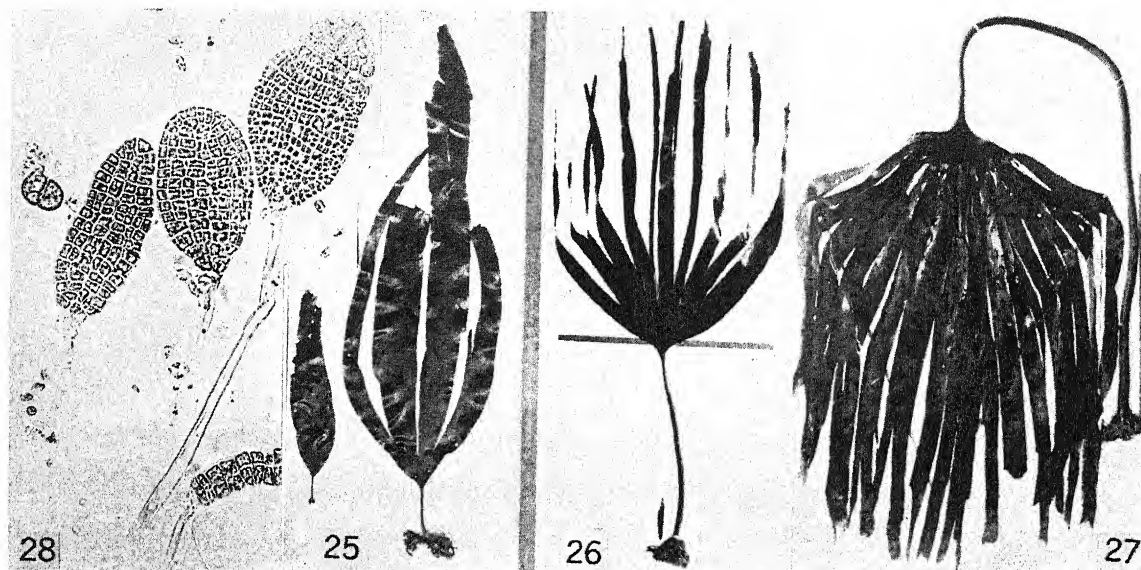


Fig. 25-28. *Laminaria pallida*.—Fig. 25-27. Stages in development of macroscopic phase of sporophyte.—Fig. 28. Young sporophytes of plants cultured in sea water. Fig. 25, about 1/12 nat. size. Fig. 26, about 1/33 nat. size. Fig. 27, about 1/30 nat. size. Fig. 28, $\times 200$.

destined to form the stipe. The sporophytes shown in figures 20 and 24 had already become distromatic in the basal region.

The development of gametophytes and young sporophytes described above is that which occurs during spring and summer. During winter months the gametophytes are considerably slower in attaining sexual maturity. This is in agreement with the findings of several other investigators of the Laminariales.

LAMINARIA PALLIDA GREV. EX J. AG.—*General remarks.*—The binomial *Laminaria pallida* is a MS. name of Greville under which the species was first described by J. Agardh in 1848.

The known South African distribution of *L. pallida* extends from the southwestern part of the Cape Peninsula to Port Nolloth (Bright, 1938) in the northwestern part of the Cape Province. The species increases in relative frequency towards the north (Isaac, 1937b; Bright, 1938), in which direction there is a gradual decrease in sea temperatures. The absence of the species along the east coast of the Cape Peninsula and points farther east is undoubtedly associated with the higher sea temperatures that

species which was described by La Pylaie in 1824 as *Laminaria ochroleuca* (cf. Hamel, 1931-1939, p. 303).

In the juvenile state *L. pallida* consists of a simple blade (fig. 25). The blade splits longitudinally at an early stage into several deep segments (fig. 25, 26) which, as growth continues, in turn become divided. In the mature state (fig. 27), the plant may attain a length of about 5 meters. Fertile plants were obtained during all months of the year but March, for which month the writer has no data. The sporangia form extensive sori on both surfaces of the segments of the blade. The histology of the sporophyte of *L. pallida* has been studied by Dangeard (1936).

Development of gametophyte.—In structure and behavior the zoöspores agree with those of *Ecklonia*. Stages in the development of the gametophytes and sexual organs are shown in figures 29 to 36. In *Laminaria pallida* the spore case is but seldom cut off by a cross wall from the germ tube, as in *Ecklonia* (compare fig. 4 to 6 and 31, 32). The plantlets do not usually become fertile until they are at least one month old; but at times, as shown in figure 34, sexual

organs develop on younger gametophytes. In older plants the antheridia occur in clusters, as in *Ecklonia*. The oogonia develop, as in *Ecklonia*, from intercalary as well as terminal cells of the female gametophytes (fig. 35).

Development of sporophyte.—The early stages in the development of the sporophyte will be seen from figures 37 to 42 and 28. In many sporophytes of the stage shown in figure 42, or even in less advanced ones, the basal cell will have formed the first rhizoid. Additional rhizoids are formed later from

coming fertile. The gametophytes in the sea water cultures were much smaller, however, than those in the nutrient solution. The sporophytes, on the contrary, appeared normal as will be seen from those of *L. pallida* shown in figure 28. The further development of the sporophytes was soon arrested, however, and they finally died without having attained as advanced a stage as did those developing in nutrient solution. The early development of reproductive organs in sea water cultures is undoubtedly owing to a starvation effect.

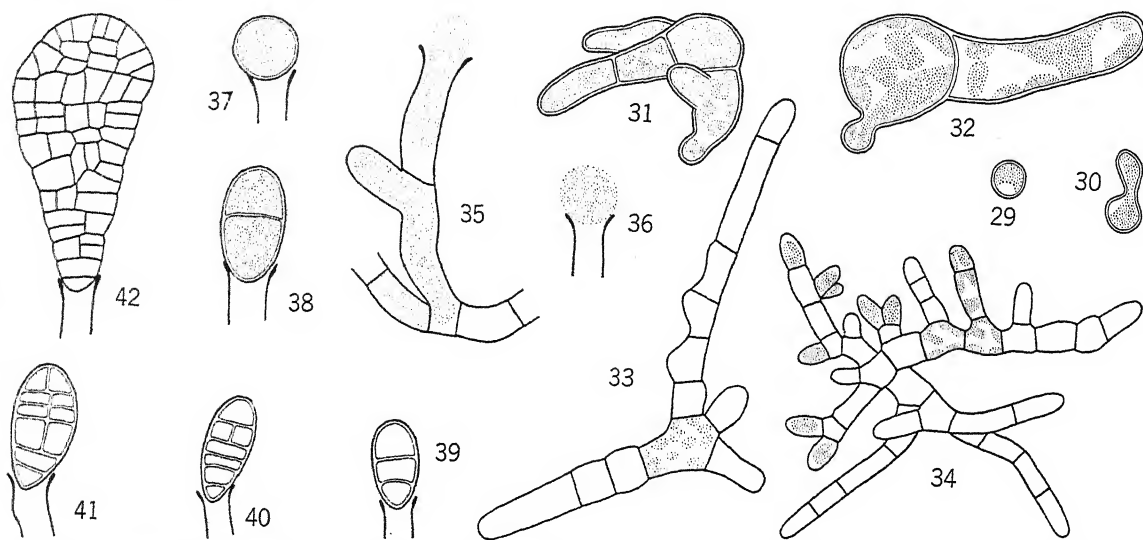


Fig. 29-42. *Laminaria pallida*.—All figures are camera lucida drawings of plants grown in nutrient solution.—Fig. 29. Rounded zoöspores, $\times 990$.—Fig. 30. Gametophyte, 2 days old, $\times 990$.—Fig. 31. Same, σ , 14 days old, $\times 990$.—Fig. 32. Same, ρ , 14 days old, $\times 990$.—Fig. 33. Same, ρ , 25 days old, $\times 440$.—Fig. 34. Same, σ with antheridia, 25 days old, $\times 650$.—Fig. 35. Portion of female gametophyte with two oogonia, one in the process of extrusion, $\times 440$.—Fig. 36. Extruded egg, $\times 440$.—Fig. 37. Zygote, $\times 440$.—Fig. 38-42. Early stages in development of sporophyte, $\times 440$.

other cells in the basal region. As in *Ecklonia*, the rhizoids are nonseptate and but rarely branched. The young sporophyte shown in figure 42 had become distromatic throughout its basal half.

In connection with the development of gametophytes and young sporophytes, it is of interest to note that Harries (1932, p. 925), working on *Laminaria digitata*, *L. saccharina*, and *L. Cloustoni*, found that "... no growth occurs where the initial supply of sea water is not renewed and nutrient materials are not supplied." In the present study it was found, however, that the gametophytes of both *Laminaria pallida* and *Macrocystis pyrifera* will become fertile in sea water alone. Thus, for example, on October 29, 1935, four cultures were made of zoöspores from the same parent plants of *L. pallida* and *M. pyrifera*. In two of the cultures the fluid consisted of sea water only while nutrient materials were added to the other two. The vessels were placed next to one another some distance away from a window and the fluid was not changed. In the sea water cultures, sporophytes had been formed in both species after twenty-five days (November 23, 1935), while the gametophytes in the nutrient solution as yet showed no sign of be-

coming fertile. In the course of this study it was found that in certain nutrient solution cultures of all three species, the gametophytes form but few reproductive organs or remain entirely sterile. Thus, a culture of *L. pallida* that was started on November 20, 1935, was still growing actively on December 3, 1936, when it was discarded, without having become fertile. Other cultures made during the same month and year formed reproductive organs. In another culture of *L. pallida* that was started on May 14, 1936, a few sporophytes had developed after twenty-seven days (June 10) but no additional sex organs were formed, and the culture was finally discarded on December 3, 1936. In none of these cultures was the nutrient solution renewed; only a small amount of distilled water was added from time to time to replace the water lost by evaporation.

These results are comparable to those obtained by Hollenberg (1939, p. 38), who states: "When gametophytes of *Eisenia arborea* in the cultures once take on an elongate sterile form, I have been unable to induce the formation of sex organs, although I have tried low temperatures ($2-3^{\circ}\text{C}.$) and changes

in light intensity as well as changes in nutrient concentration."

MACROCYSTIS PYRIFERA (L.) C. AG.—*General remarks*.—This plant has attracted the attention of naturalists and navigators since early times (cf. Hooker, 1845, p. 157). The species was described by Linnaeus in 1771 from specimens that were collected by König on his voyage to India. The source of König's plants, as given by Linnaeus, is "*Mare Aethiopicum*." Setchell (1932) attributes this name to certain Antarctic islands, but Papenfuss (1940) recently pointed out that according to old maps it applies to that part of the Atlantic which lies opposite Africa from the coast of Guinea southward.

fera in South Africa, it becomes evident that the area of distribution is limited to a comparatively small region lying approximately between latitudes 33° 24'S. and 34° 8'S. According to data presented by Isaac (1937a), the minimum and maximum annual sea temperatures of this region may be taken as varying between approximately 12° and 16°C. In consideration of the fact that in Antarctic regions *M. pyrifera* thrives at temperatures much lower than these (cf. Setchell, 1932, p. 446) and that along the west coast of South Africa there is a gradual decrease in sea temperatures from south to north, it would be expected that the species would extend northward for a considerable distance beyond Das-

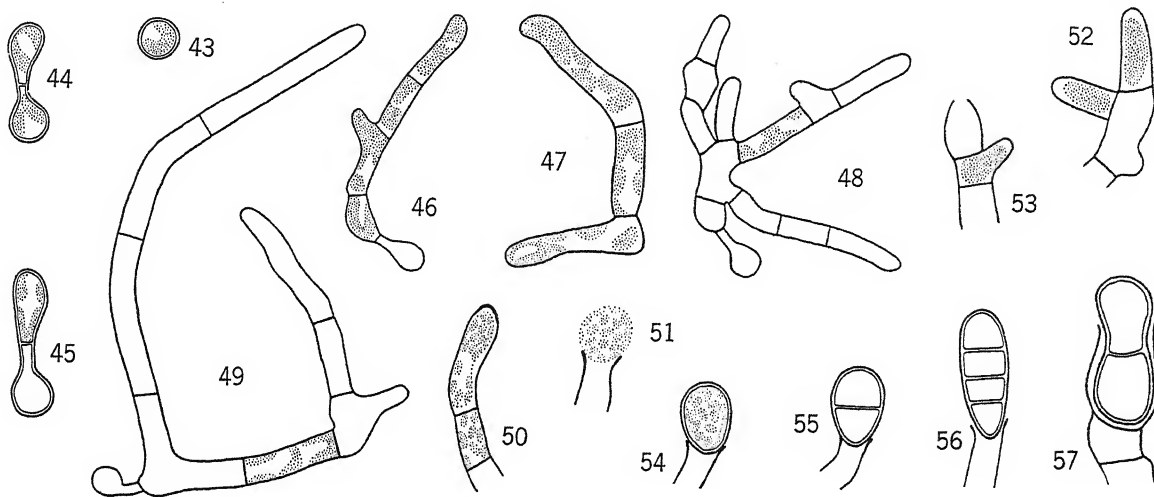


Fig. 43-57. *Macrocystis pyrifera*.—All figures are camera lucida drawings of plants grown in nutrient solution with the exception of figure 57, which is of a plant grown in sea water.—Fig. 43. Rounded zoospore, $\times 990$.—Fig. 44. Gametophyte, 5 days old, $\times 990$.—Fig. 45. Same with spore case emptied of cytoplasm, 5 days old, $\times 990$.—Fig. 46. Same, δ , 17 days old, $\times 650$.—Fig. 47. Same, ϕ , 17 days old, $\times 650$.—Fig. 48. Same, δ , 25 days old, $\times 650$.—Fig. 49. Same, ϕ , 25 days old, $\times 650$.—Fig. 50. Immature oogonium, $\times 440$.—Fig. 51. Extruded egg, $\times 440$.—Fig. 52, 53. Antheridia, $\times 990$.—Fig. 54. Zygote, $\times 440$.—Fig. 55-57. Early stages in development of sporophyte, figure 57 showing a sporophyte developing within the oogonium from an unextruded egg, $\times 440$.

As is well known, *Macrocystis pyrifera* has an extensive distribution, which has been discussed and mapped by Setchell (1932). As will be seen from Setchell's account this area is inhabited in part by a second species of *Macrocystis*, *M. integrifolia* Bory.

The South African distribution of *M. pyrifera* is limited to the southwestern part of the Cape Province, extending from the Olifants River in the north to Cape Point in the south (i.e., approximately between latitudes 31° 42'S. and 34° 20'S.). It is likely, however, that the Olifants River record (Drège, 1843, pp. 107, 200) is based on a specimen that was cast ashore, in which event the northernmost known record of occurrence would be Dassen Island (Isaac, 1937b, p. 142). The southernmost locality, Cape Point (Delf and Levyns, 1926, p. 503), also requires confirmation. The species does, however, occur at Slangkop, a station in the central west coast of the Cape Peninsula. Assuming then that Dassen Island and Slangkop are the northernmost and southernmost limits of occurrence, respectively, of *M. pyri-*

sen Island. As yet there is no evidence, however, to support this supposition.

In South Africa *M. pyrifera* is a shallow water plant, inhabiting localities that are protected from the full force of the waves. The plants are much shorter than those recorded from certain other parts of the world and probably never exceed 15 meters in length, a dimension in excess of any of the specimens seen by the writer.

The morphology of the sporophyte of *M. pyrifera* has been the subject of a number of studies, the most important recent ones being those of Skottsberg (1907) and of Setchell (1932).

Fertile plants were obtained by the writer during all months of the year with the exception of April and June. This gap is filled, however, by Delf and Levyns (1926), who obtained fertile specimens during April, and by Levyns (1933), who secured some in June.

In South African plants, the sori seem to be confined to the smooth or but slightly wrinkled pinnae of

the basal short branches, whereas in the Falkland Islands, Skottsborg (1907) observed sori also on the wrinkled pinnae of long branches. The sori are formed on both surfaces of the pinnae and may occupy extensive areas or occur as isolated patches.

Development of gametophyte.—The development of the gametophyte and the early stages of the sporophyte have been studied previously by Brandt (1923), Delf and Levyns (1926), and Levyns (1933). None of these studies was complete, however, and certain statements required substantiation. Brandt misinterpreted the oogonium and referred to it as a proembryo.

In structure and behavior the zoöspores agree with those of *Ecklonia*. According to Delf and Levyns

As stated in the section on *Laminaria pallida*, the gametophytes when cultured in sea water become sexually mature within twenty-five days. Levyns (1933) obtained young sporophytes in a sea water culture that was seventeen days old (June 8–25). Gametophytes grown in nutrient solution are slower in attaining sexual maturity. In such cultures the writer has observed the differentiation of oogonia thirty-seven days after the culture was started (during spring), while in a culture that was forty-four days old reproductive organs were abundant (fig. 58) and sporophytes had been formed.

Development of sporophyte.—The development of the young sporophyte (fig. 54–56, 59) is in accordance with that of other members of the order. In

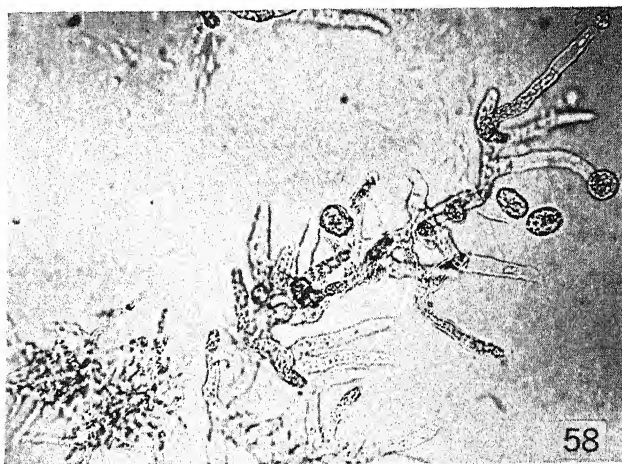
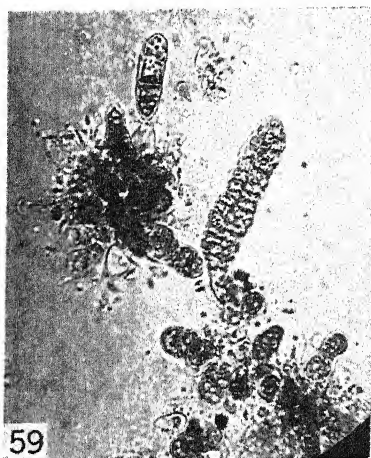


Fig. 58, 59. *Macrocyctis pyrifera*.—Fig. 58. Male and female gametophytes, the latter with extruded eggs, of plants cultured in nutrient solution, 44 days old, $\times 200$.—Fig. 59. Gametophytes and young sporophytes of plants cultured in sea water, 25 days old, $\times 200$.

(1926), the zoöspores of *Macrocyctis pyrifera* appear to be of two sizes; but the writer, in agreement with Levyns (1933), found them to be of the same size.

Stages in the development of the gametophytes and sexual organs are shown in figures 43 to 53 and 58. In the majority of gametophytes the spore case is emptied of cytoplasm, as shown in figure 45, but not infrequently this cell remains functional (fig. 44). The oogonia and antheridia are formed as in *Ecklonia*.

The statements of Delf and Levyns (1926) regarding the sexual nature of the gametophytes are contradictory. In their figures 1a and 1b are shown plantlets that were believed to be male and female gametophytes, respectively, while on page 505 they state: "The empty thick-walled cells at the base of the young sporophyte (fig. 2) seem to indicate a bisexual gametophyte. . . ." These authors obviously mistook empty oogonia for empty antheridia. Brandt (1923) makes no reference to male gametophytes, while Levyns (1933) describes and figures them correctly.

figure 57 is shown a two-celled sporophyte which was formed from an egg that had not been extruded from the oogonium. The retention of the ripe egg in the oogonium is an abnormal condition that was frequently observed in plants cultivated in sea water only and is probably associated with the general lack of vigor of such plants. In this connection attention may be drawn to the statement of Levyns (1933, p. 352) that: "In *Macrocyctis* the egg is fertilized within the oogonium, and the process of extrusion takes place *after* the zygote has been formed." The writer's observations indicate that Levyns, who had sea water cultures only, was misled in believing that the retention of the ripe egg in the oogonium represents the normal condition in *Macrocyctis*. Moreover, her conclusion that extrusion occurs after fertilization is probably based, judging from her figures, on observations of different eggs—some that were not extruded, some that were partially extruded, and others that were completely extruded. The writer's sea water cultures showed similar stages but the presence of young sporophytes within the oogonia (fig. 57) indicates that an unextruded egg remains in the oogonium even

after fertilization. With respect to the undischarged eggs, it is highly probable, however, that a rupture of the oögonial wall precedes fertilization and that the spermatozoid enters through this break. The body lying next to the partially extruded egg in Levyns' figure 8 probably does not represent the remains of the spermatozoid, as the author believes, at least not the one which fertilized the egg, since it has been shown by McKay (1933, pl. 14, fig. 2) and by Hollenberg (1939, fig. 22–25) that the spermatozoid completely coalesces with the egg during fertilization.

Finally, it may be noted that *Chorda Filum* is the only member of the Laminariales in which the ripe eggs are known to be normally only partially extruded from the oögonium (Kylin, 1933; Kanda, 1938). *Chorda*, however, differs from other Laminariales also in several other features, such as in the formation of hairs and of septate rhizoids by the young sporophytes.

SUMMARY

Ecklonia maxima, *Laminaria pallida*, and *Macrocystis pyrifera* inhabit the colder west coast waters of South Africa, *E. maxima* being the only species

of the three that extends eastward beyond the Cape of Good Hope.

The geographical distribution of *E. maxima* and *L. pallida* is discussed, and it is pointed out that St. Paul Island in the southern ocean is probably the only authentic extra-South African station for these species.

Fertile sporophytes of the three species were secured during all seasons of the year. The development of the gametophytes and the young sporophytes is similar to that described for other Laminariales.

In agreement with the observations of Levyns (1933) and in contrast to those of Delf and Levyns (1926), it was found that the zoöspores of *Macrocystis* are not of two distinct sizes.

In nutrient solution cultures the eggs of *M. pyrifera* are extruded from the oögonia, indicating that the retention of the egg in certain oögonia of plants cultured in sea water only is an abnormal condition and not a characteristic feature of this species, as was believed by Levyns (1933).

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PARASITISM AMONG THE CHYTRIDS¹

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FUNGUS PARASITES of other fungi are fairly common in nature, but parasitism between closely related genera and species is comparatively rare. This is particularly true in the order of simple fungi known as the Chytridiales where only a few cases of parasitism have been found. Up to the present time only nine species parasitic on other chytrids have been reported, as is shown by the following list:

Olpidiopsis (?) *Sphaeritae* comb. nov. on *Sphaerita endogena* (Dangeard, 1889).

Pleotrachelus paradoxus on *Rhizophidium distinctum* (Petersen, 1905).

Tylochytrium dangeardii comb. nov. on *Saccomyces dangeardii* (Serbinow, 1907).

Phlyctocytrium Synchytrii on *Synchytrium endobioticum* (Köhler, 1924).

Rozella Polyphagi on *Polyphagus Euglenae* (Sparrow, 1936a).

R. maximum on *Chytridium Polysiphoniae* (Sparrow, 1936b).

Rozella sp. on *Rhizophlyctis rosea* (Ward, 1939).

R. Cladochytrii on species of *Cladochytrium* and *Nowakowskiella* (Karling, 1941).

R. Endochytrii on *Endochytrium operculatum* (Karling, l.c.).

All of these species are parasitic on chytrids, which in turn parasitize amoebae, algae, and higher plants or live saprophytically in dead vegetable debris. Of the nine species listed above, none attack hosts which belong to the same genus as the parasite, and only one, *T. dangeardii*, occurs on a host within the same family. Two of the parasites are extramatrical, eucarpic, and rhizidiaceous, while the remainder are intramatrical, holocarpic, and *Olpidium*-like. To this latter group may now be added another species of *Rozella* which has recently been found in *Rhizophlyctis petersenii*.

Rozella is of particular interest to pathologists because all of its species are parasites of higher Oomycetes and chytrids and cause local hypertrophy or septation of the host hyphae. Although species of this genus have been recognized for more than a half century, very little is known about their development within the host. Nonetheless, students of *Rozella* divide the species into two groups on the basis of their development within the host and the reactions induced. Species in the so-called "sporangium-group" cause marked local hypertrophy of the host hyphae, and each thallus forms a single sporan-

gium or resting spore. In the "septigena-group" the thallus or plasmodium is reported to segment and give rise to a linear series of sporangia which mature in basipetal succession. The presence of the parasite also causes slight hypertrophy and leads to the formation of cross walls in the host hyphae, whereby the successive sporangia are separated from each other. However, no conclusive cytological data have been presented to support these distinctions. Cornu (1872) reported that after entry into the host the zoospores become indistinct and obscure but form a plasmodium in the center of host hyphae. While he found no conclusive evidence to support his view, he nevertheless believed it more plausible that the successive sporangia in *R. septigena* originate by fragmentation of the plasmodium than from multiple infection in basipetal succession. Fischer (1882) asserted that in the *R. septigena*, the type species of the "septigena-group," the parasite loses all individuality as its protoplasm mixes with that of the host and forms a plasmodium, which, however, eventually consumes the host constituent. At maturity this plasmodium divides into a number of portions which become permanently separated through the formation of cross septa by the host. Later, each portion forms one sporangium which completely fills the delimited segment of the host hypha. While it is true that the parasite usually becomes largely invisible in the relatively dense protoplasm of the host and gives the appearance of having fused with the latter, neither Fischer nor subsequent workers have clearly demonstrated its miscibility with the host protoplasm. Dangeard's (1890) figures of thalli from fixed, sectioned, and stained material, however, suggest that the host protoplasm is consumed completely by the time the thalli are mature.

Likewise, it is not certain whether the plasmodium develops from a single zoospore or is formed by the union of several protoplasts within the hosts. Butler (1907) upheld the latter view from his study of species in the "sporangium-group" but did not support it by cytological data. Furthermore, the development of the wall around the mature parasite in both groups is not clearly understood. Following Cornu's early description of the process, all students of *Rozella* have reported that as the sporangium wall of the parasite develops, it becomes fused so closely with that of the host that the two are indistinguishable and cannot be separated. While this type of development seems to occur, its successive stages have never been described or figured. In addition, our knowledge

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of the nutrition of *Rozella* is limited and confusing. As the parasite matures, masses of granules and bodies appear in the central vacuole which look like extraneous food or waste material extruded by the protoplasm in dedifferentiating for sporogenesis. The presence of such material suggests that *Rozella* is capable of engulfing and feeding on masses of host protoplasm and, like *Woronina glomerata* and protoomyxean species, discards the accumulated waste products before forming zoospores or resting spores. If this occurs, it is obvious that the nutrition of *Rozella* differs radically from that of other genera of the family Olpidiaceae in which it is now included.

From the taxonomic and systematic standpoints, also, *Rozella* has been greatly confused. Cornu described the zoospores as posteriorly uniflagellate in both groups, but later in *R. septigena* and *R. simulans*, Fischer figured them as biflagellate and heterocont. Fischer was thus confronted with *Rozella* species of two types, but he (1892) solved this difficulty to his satisfaction by erecting a new genus, *Pleolpidium*, for the "sporangium-group" species with posteriorly uniflagellate zoospores and retained Cornu's *Rozella* for the biflagellate, heterocont, "septigenous" species. It is to be noted and emphasized in this connection that Fischer's (1882) statement concerning the number, relative lengths, and position of the flagella does not relate specifically to *R. septigena* and *R. simulans*. It was applied in general to the zoospores of *Olpidiopsis*, *Woronina*, and *Rozella* as a group, and Fischer thus asserted that the zoospores of these genera are exactly alike in this respect. *Pleolpidium* was accordingly included in the family Monolpidiaceae (Olpidiaceae), while *Rozella* was placed among the Merolpidiaceae (Synchytriaceae). Fischer's disposition of these genera was accepted by Schroeter (1897), but Minden (1911), Gaumann (1926), Gaumann and Dodge (1928), and Fitzpatrick (1930) shifted *Rozella* to the family Woroninaceae and placed it next to *Woronina*. The inclusion of *Rozella* in the Woroninaceae was generally accepted by chytridologists until Foust (1937) discovered a septigenous *Rozella* species with posteriorly uniflagellate zoospores in *Allomyces arbuscula*, and, in 1938, Sparrow proposed the retention of *Rozella* in the original sense of Cornu and the suppression of *Pleolpidium* to the status of a synonym of *Rozella*. Nonetheless, there still remains the question of Fischer's biflagellate *R. septigena* and *R. simulans*. Fischer's observations on the occurrence of this species and the number and relative lengths of the flagella were later confirmed by Minden (1911) and Tokunaga (1933) for *R. simulans*. The latter worker, however, figured and described the two unequal flagella as being anterior instead of lateral in position, which raises the question of whether or not he was studying Fischer's species. In the event that Fischer, Minden, and Tokunaga were correct in their observation, it will be necessary to segregate these species in a new genus. Further complications have arisen by the discovery of parasitic species which resemble very closely members of the "sporangium-

group" of *Rozella* but which also possess biflagellate zoospores. Butler (l.c.) and Miss Waterhouse (1940) described them as being *Pleolpidium*-like but did not dispose of them taxonomically. Obviously, provision must be made for these species also.

These are some of the outstanding problems which remain to be solved and which make *Rozella* particularly intriguing to cytologists, pathologists, and systematists. The solution of many of these problems depends largely upon cytological study of fixed, sectioned, and stained preparations, and while such studies on *Rozella* may seem relatively simple and easy, they involve unusual difficulties. In the first place, the parasite is very minute in the early developmental stages, and secondly, it usually does not occur in epidemic abundance and thus provide sufficient material for fixing, embedding, and staining. Furthermore, except for the higher Oomycetes, its hosts are usually short-lived, and it is, therefore, extremely difficult to keep the parasite in culture.

In view of the many problems which remain unsolved in relation to *Rozella*, a more intensive study was undertaken of *R. Cladochytrii* and *R. Endochytrii*, two parasites of chytrids which have been previously described by the author (1941) in a brief paper on Texas chytrids. The present paper is the result of this study and relates primarily to the host range, pathogenicity, and development of these species.

ROZELLA CLADOCHYTRII.—*Host range and effect on hosts.*—This species was first observed in the sporangia and intercalary enlargements of the rhizomycelium of *Nowakowskiella profusum* which was growing on cellophane in charcoal water. As soon as its parasitic nature was recognized, inoculation experiments were started to determine its host range and pathogenicity to other chytrids and filamentous fungi. Fortunately at this time, the author had on hand pure and mixed cultures of several chytrids, and it was comparatively easy and simple to test their susceptibility to the parasite. Bits of corn leaves, cellophane, and hemp seeds were inoculated with the fungi to be tested and were placed in Petri dishes containing charcoal water. To these cultures were subsequently added fragments of cellophane and corn leaves containing the parasitized rhizomycelium of *N. profusum*.

Microscopic examinations of these cultures were made daily, and within a period of six to eighteen days *R. Cladochytrii* had infected *Nowakowskiella ramosum*, *N. elegans*, *Cladochytrium replicatum*, *C. hyalinum*, and *C. crassum*. On the other hand, *Endochytrium operculatum*, *Rhizophlyctis petersenii*, *Rhizophidium globosum*, *Olpidium gregarum*, *Catenochytridium carolineanum*, *Diplophlyctis intestina*, *Entophlyctis heliomorpha*, *E. texana*, *Saprolegnia* sp., *Achlya* sp., and *Pythium de Baryanum* were not attacked and have remained healthy. Of the susceptible hosts only *N. profusum* and *C. replicatum* were severely parasitized. A few infected sporangia and intercalary swellings of *N. elegans*, *N. ramosum*, *C. hyalinum*, and *C. crassum* were found, but in no in-

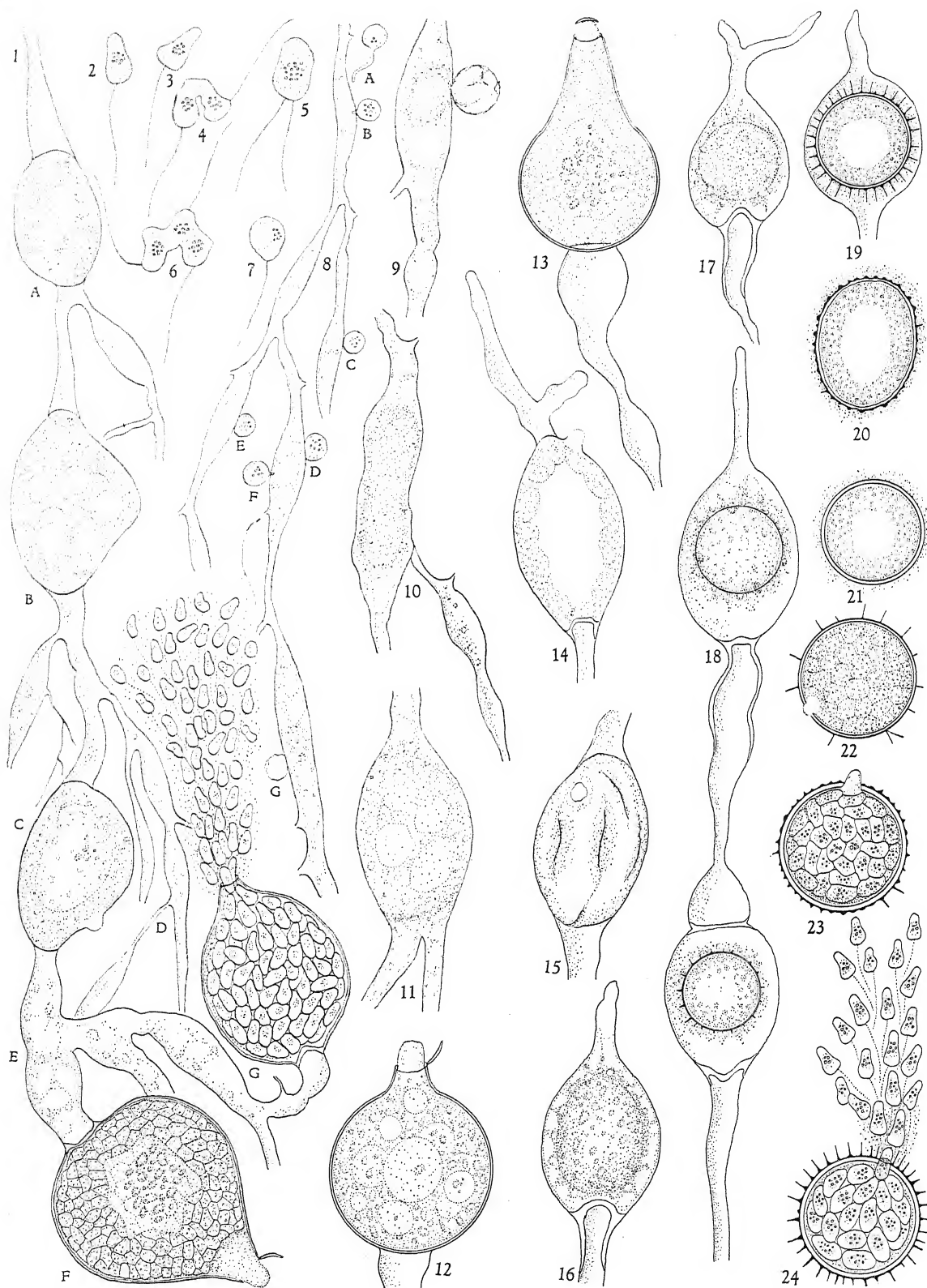


Fig. 1-24. *Rozella Cladochytrii*.—Fig. 1. Portion of infected rhizomycelium of *N. profusum* with thalli and sporangia of *R. Cladochytrii* at A, B, C, F, and G.—Fig. 2, 3. Obclavate zoospores.—Fig. 4, 5. Biflagellate zoospores.—Fig. 6. Tri-

stance did the infection attain epidemic proportions in these hosts. Present data accordingly suggest that *R. Cladochytrii* may be limited in host range to saprophytic species of the Cladochytriaceae. Unfortunately, species of *Catenaria*, *Urophlyctis*, *Physoderma*, etc., were not available for infection tests.

The effect of *R. Cladochytrii* on its hosts varies considerably. In the case of *N. profusum* infected sporangia and intercalary enlargements may be only slightly hypertrophied or up to 200 per cent larger than normal. The tenuous portion of the rhizomycelium immediately adjacent to infected sporangia may also become slightly or greatly enlarged, as is shown at D and E, respectively, in figure 1. In another instance the hypertrophied portion measured 25 μ in diameter, although it was not directly parasitized. While the hypertrophy and distortion is usually localized, in exceptional cases the entire rhizomycelium may be involved and become hypertrophied. *Rozella Cladochytrii* differs thus from other known species of the genus by its ability to cause hypertrophy of cells and organs which are not directly parasitized. Septation of parasitized filaments in the region of infection, such as is caused by *R. septigena* and *R. Allomyces*, has never been observed in our material. No cases of marked hypertrophy of the rhizomycelium of *N. ramosum*, *N. elegans*, *C. hyalinum*, and *C. crassum* have been found. In these species, however, infection was limited, and it was accordingly difficult to determine how extensive and conspicuous hypertrophy may become in these hosts. In *C. replicatum*, on the other hand, the effect of the parasite was quite evident. The degree of hypertrophy was in general slight, but thickening of the cross septa and lateral walls at the base of infected sporangia and spindle organs (fig. 17-19) frequently occurred. In some cases these thickenings were as marked as those caused by *R. Rhipidii* and *R. Apodyae* (Cornu, 1872) in their respective hosts. In addition, the exit tubes of infected sporangia often become elongated, branched, and distorted (fig. 15, 18), while the formation of golden-red refringent globules in the sporangia, characteristic of this species, is usually completely inhibited. As a result, parasitized thalli are hyaline and appear markedly different from normal ones.

Infection.—Infection occurs most abundantly when the rhizomycelium is young and growing vigorously, but it may also take place when the host is comparatively old. While infection of full-grown sporangia has not been observed, it probably occurs, because parasitized sporangia with fully developed opercula may often be found. *Nowakowskiella profusum* is more favorable than the higher Oomycetes for the study of infection and development of the parasite. When thin flakes of cellophane or lens

paper are used as substrata, the rhizomycelium grows out into the surrounding water, where infection may be studied under very high magnifications. Furthermore, the tenuous filaments are relatively small in diameter and usually contain a sparse amount of vacuolate protoplasm, which does not obscure the parasite as completely as in species of *Allomyces*, *Rhipidium*, etc.

In spite of these advantages, however, the author has been unable to solve the fundamental problems in relation to the development of *Rozella* which were enumerated in the introduction. While my observations lead me to believe that no true amoeboid plasmodium is present in the non-septigenous species of *Rozella* and that each infection gives rise to a single thallus or sporangium, proof of these beliefs must await cytological study of fixed and stained preparations.

The zoospores of *R. Cladochytrii* come to rest on the host (fig. 9), round up, and within a period of two to five hours put forth a narrow, short germ tube which penetrates the host wall (fig. 9B, 9F). While a large number of rounded-up zoospores may be found on the rhizomycelium, all do not germinate and infect the host. According to my observations, the majority degenerate. The content of germinating spores slowly flows into the host and may become partly or largely obscured, depending on the density and optical heterogeneity of the host protoplasm in the region of infection. The extramatrical, empty zoospore case shrivels up after germination and soon disappears (fig. 9G, 10). Zoospores may occasionally round up and encyst apart from the host in the same manner described by Miss Waterhouse (1940) for a parasite on *Phytophthora*. Such zoospores have never been seen to resume motility or exhibit diploplanetism in *R. Cladochytrii*, but in two instances they developed germ tubes (fig. 9A) which grew to be 8 and 14 μ long and later degenerated.

After entering the tenuous portions of the rhizomycelium, the young parasite may be recognized under favorable circumstances as a denser globular region (fig. 9G, 10) in the rhizomycelium. Even under the best of optical conditions its outline is partly obscured by the host protoplasm and never appears sharp and distinct. No visible antagonism between the two protoplasts has been observed in the initial stages of infection. As the parasite enters, a very slight local agitation of the host protoplasm occurs momentarily, but no streams or circulatory currents are visible. Very shortly after infection, however, the protoplasm appears to become denser around the parasite as if actual accumulation occurs at the region of entry. This appearance, on the other hand, may be due in part to the opacity of the parasite itself. Later, visible effects of the presence of the

flagellate amoeboid zoospore.—Fig. 7. Zoospore at close of motile period.—Fig. 8. Infection of rhizomycelium.—Fig. 9-11. Developmental stages of parasite in rhizomycelium of *N. profusum*.—Fig. 12. Multivacuolate stage of parasite in sporangium of *N. profusum*.—Fig. 13. Later stage in sporangium of *N. elegans*.—Fig. 14. Cleavage (?) of parasite's protoplasm in sporangium of *C. replicatum*. All obstructing extraneous material omitted from drawing.—Fig. 15. Empty shrunken sporangium of *R. Cladochytrii*.—Fig. 16-19. Stages in resting spore development.—Fig. 20, 21. Warty and smooth resting spores.—Fig. 22-24. Stages in resting spore germination and emergence of zoospores.

parasite become apparent in the host protoplasm. It becomes more vacuolate and coarsely granular and assumes a faint yellowish gleam. The decrease in degree of dispersion of the granular refractive material is very characteristic and leads to the formation of globular, elongate, and irregular gleaming particles or masses (fig. 11, 12). In the early stages of infection no hypertrophy is visible, and in regions where it occurs it is not noticeable until fifteen to twenty hours after infection.

The parasite appears to lack a definite structural membrane in the early developmental stages (fig. 10-12), but it is doubtless immiscible with the host protoplasm. While I have no direct cytological data to support this view, I question the assertion that the parasite loses its identity, infiltrates the interstices and vacuoles, and mixes with host protoplasm as Fischer and Foust have reported for *R. septigena* and *R. Allomyces*. No amoeboid movement of the young thallus has been observed, although at times it may be elongate and slightly irregular in outline. As the parasite grows and increases in size, numerous small vacuoles appear where it is located in the host (fig. 11, 12) in the manner described by Cornu, Fischer, and Foust for other species of *Rozella*. Fischer in particular speaks of these vacuoles as relating to the host protoplasm, but my observations lead me to believe that they belong to the parasite, as is suggested in figures 11 and 12. The fact that they become fewer in number and larger in size and eventually form a large central vacuole in the mature sporangium of the parasite supports this view. Granules and globular masses of various sizes and shapes are usually present in the large vacuoles and undergo active Brownian movement, as Fischer and Foust have described in *R. septigena* and *R. Allomyces*. In the latter species, however, Foust described them as clumps of host protoplasm undergoing digestion in the vacuoles. In the mature stages of *R. Cladochytrii*, these granules and masses often have the appearance of extraneous food material which has been extruded into the central vacuole before sporogenesis.

The successive developmental phases of the parasite are difficult to follow in sporangia of cladocytriaceous hosts because they are usually obscured in the early stages by the surrounding protoplasm. Infection may occur before or after the sporangia are delimited by cross septa, but usually before they are full grown and mature. The presence of the parasite in older sporangia apparently does not arrest development at once, because normal and fully developed opercula (fig. 1F, 12, 14) have frequently been found on infected sporangia. Parasitized sporangia may be readily distinguished from normal ones and are easily recognized by their vacuolate, dense, coarsely-granular, gleaming contents. Here it is even more difficult to determine which part or if all of the protoplasm relates to the parasite. As the latter matures, however, it fills the sporangium completely and apparently develops a distinct wall which, nonetheless, is impossible to distinguish clearly from that

of the host. In intercalary swellings of the rhizomycelium, on the other hand, the wall appears distinctly at the ends of the oval and elongate thalli (fig. 1A-C). Before, during, or after the wall is formed, the small vacuoles fuse into one or two larger central ones (fig. 1C, 14), while the remainder of the protoplasm, apart from the large granules and bodies, becomes more finely and evenly granular in appearance. The large vacuoles so formed may be uneven in outline and undergo changes in shape and position.

By this time the exit papillae have been formed. Usually only one is present, but as many as three have been found on one sporangium. In sporangia of *Nowakowskiella*, they push off the operculum if one is present and project out of the short neck (fig. 1F, 13, 14). In size, the papillae vary from 2 to 3.5 μ across and 3 to 5 μ high. They are hyaline and optically homogeneous in contrast to the granular protoplasm in the sporangia. Cleavage of the protoplasm into zoospores appears to be progressive and largely centrifugal instead of simultaneous as reported by Fischer for *R. septigena*. In large sporangia the early stages of segmentation are difficult to interpret, but in smaller ones the scalloped border of the central vacuole suggests the presence of cleavage furrows. Figure 15 shows a small infected sporangium of *C. replicatum*, drawn in median section with all of the obscuring material omitted, in which sharp furrows appear to be developing from the border of the central vacuole. Presumably, as these furrows reach and cut through the plasma membrane, the fluid from central vacuole becomes dispersed among the cleavage segments, because the vacuolar region becomes indistinct and the whole mass of segments shrinks slightly. Later, the central vacuolar space becomes more clearly outlined again and remains after cleavage and the formation of the zoospores have been completed (fig. 1F). The hyaline area or fluid continuous with the exit papilla may extend for some distance down into the sporangium and has a distinct convex boundary where it impinges upon the zoospores, as is shown in figure 1F.

Within twenty to fifty-five minutes after cleavage has been completed, the zoospores begin to glide upon each other and upon the extraneous granules or bodies dispersed among them. This movement increases gradually in velocity, until the whole mass of zoospores is swirling in the sporangium. In a few sporangia in *N. profusum*, however, no movement prior to the escape of the zoospores has been observed, but such cases may be unusual. In dehiscence of the sporangia the exit papillae deliquesce slowly, and the hyaline, slimy fluid flows out and spreads in the surrounding water. In one-half to three minutes the zoospores begin to emerge in a thick stream (fig. 1G), which may run out to a distance of several hundred microns before the zoospores become motile. They are surrounded by the slimy substance which exudes with them from the sporangium. The zoospores lie quiescent for a few seconds, and as the slimy fluid becomes diluted, they jerk about a few times and soon thereafter go darting off. The remain-

ing spores in the sporangium swarm about until they reach the orifice and emerge by their own motility. If unable to escape, they remain active for twenty-eight hours before degenerating. Complete emergence of all zoospores in unobstructed sporangia may last from five minutes to three hours, depending on the size of the sporangium and the ease with which the spores reach the orifice.

A considerable amount of extraneous waste material in the form of granules, globular bodies, and slime remains in the sporangium after all zoospores have emerged. This material is undoubtedly of the same nature as that visible earlier in the large central vacuole. Within a few weeks the empty sporangium may begin to shrink and shrivel, and in such cases the separate wall of the parasite may become more evident. This is particularly clear in the case of intercalary sporangia of the type shown in figure 16.

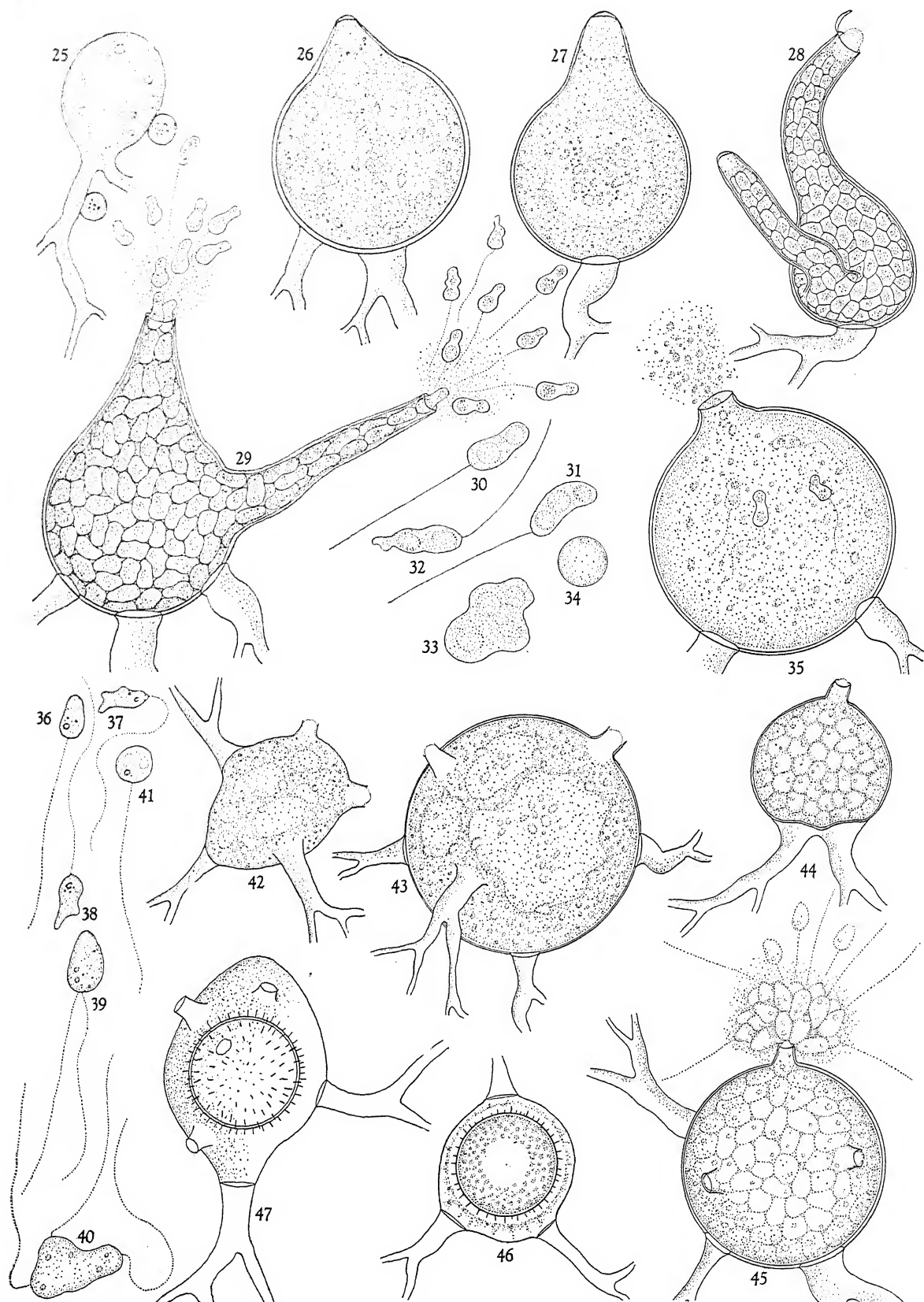
Zoospores.—The zoospores of *R. Cladochytrii* are characteristic in size, shape, and behavior and may be readily recognized in a mixed culture of chytrid zoospores. As is shown in figures 1G, 2, and 3, they are obclavate and obpyriform as they emerge from the sporangium. The tapering anterior and rounded or slightly flattened posterior ends are strikingly characteristic. Bi- and multiflagellate zoospores are not uncommon (fig. 4-6), but more than 98 per cent of the zoospores are posteriorly uniflagellate. Internally, they appear optically homogeneous except for a region in the center or slightly toward the anterior end which is occupied by several minute globules or granules. This region is not markedly refringent, but it may be readily recognized as the zoospores dart about. No zoospores with one large refractive globule have been found in *R. Cladochytrii*. The motion of the spores in swimming is jerky and darting. The zoospores may stop, suddenly reverse, and start off in another direction. They may continue swimming for one-half to two hours, and as their activity diminishes, they become more globular and look like rain drops (fig. 7). Furthermore, they become vacuolate and increase slightly in size, so that if an accurate picture of their characteristic shape and size is desired, they must be studied shortly after emerging from the sporangium.

Resting spores.—The resting spores of *R. Cladochytrii* were found in abundance in *C. replicatum* and *N. profusum*; sparsely in the other hosts. They usually appear at the conclusion of the zoosporic phase and as the culture becomes old, but zoosporangia and resting spores may sometimes be found together. The development of the spores is fundamentally similar to that described by Cornu, Fischer, Butler, and Foust for other species of *Rozella*, and nothing significantly different has been observed. Successive developmental stages are shown in figures 17 to 20. The early stages may be readily recognized by the density of the protoplasm in the center of an infected sporangium or intercalary enlargement. Later the protoplasm becomes sparse at the periphery as it accumulates around the parasite (fig. 17), so that more or less radial strands become evi-

dent. No sharply-defined membrane around the parasite is visible at this stage, but later (fig. 18) the wall may be easily seen. When viewed from above and *in toto*, the host protoplasm appears to impinge directly on the developing resting spore, but in median views it is obvious that a hyaline zone separates the host and pathogen. Such a zone or areola appears to be a constant character of spore development in *Rozella* and has been reported by all students of this genus. It is usually surrounded by a dense layer of host protoplasm from which run numerous radial streams to the periphery. It is in this hyaline zone that the echinulations on the exospore appear, which suggests that it is the region of transformation of the host protoplasm into spore wall material. In *R. Allomyces*, however, Foust reported the granules migrate bodily into this zone, arrange themselves "at right angles to the spore wall, and appear to fuse forming spines." No fusion of granules has been found in our material, but the spines and excrescences, nevertheless, appear to be formed from modified host protoplasm.

The resting spore of *R. Cladochytrii* may be smooth (fig. 22), warty (fig. 21, 24), or spiny (fig. 20, 23, 25). The spines may be long and sparse (up to $3.5\ \mu$) or numerous and shorter. A few spores have been found (fig. 24) in which spines are interspersed among warts or excrescences. A smooth wall usually indicates immaturity, but in *R. Cladochytrii* smooth spores may attain maturity and germinate without the formation of spines or warts. The wall is relatively thin, $1.5-2\ \mu$, and hyaline or faintly amber in color. The spores are predominantly spherical in shape, but oval and slightly elongate ones may also occur. They also vary considerably in size; ranging from $10-14\ \mu$ in diameter in *C. replicatum*, $13-15\ \mu$ in *C. hyalinum*, $9-13\ \mu$ in *C. crassum*, $8-22\ \mu$ in *N. profusum*, $12-16\ \mu$ in *N. elegans*, and $11-15\ \mu$ in *N. ramosum*. The protoplasm of mature spores is coarsely but more or less evenly granular with a large, slightly irregular vacuole in the center. This clear central region has been described by other workers as a large globule of hyaline substance, but in our species it appears to be vacuolate.

Germination may begin within six weeks after the resting spores are formed. By this time the host cell has partially degenerated and freed the spores, but the latter may germinate while the host sporangium is still intact. In this process they are transformed directly into zoosporangia and produce posteriorly uniflagellate zoospores in the manner described by Foust for *R. Allomyces*. While smooth and spiny resting spores have often been found associated with and were generally believed to relate to *Rozella* parasites, Foust was the first to observe their germination and thus prove conclusively their direct relationship. The initial germination stages are difficult to detect in *R. Cladochytrii*, but in the process the protoplasm becomes more finely granular and the central vacuolate region less distinct in outline. A pore is soon formed from which an exit papilla projects to the outside. The latter may be



low and inconspicuous (fig. 23) or fairly high and tapering (fig. 24). The protoplasm cleaves into segments (fig. 23) which eventually begin to glide upon each other and move slowly about. Shortly before the exit papilla deliquesces, the movement becomes increasingly rapid, until the entire mass is swirling about. This whirling motion and subsequent stages of zoospore behavior are similar to those described previously for the zoosporangia. As the exit papilla deliquesces, a mass of slime exudes and spreads in the surrounding water, and in a short while the zoospores emerge. These likewise lie quiescent for a few seconds in the slimy material, jerk about a few times, and then dart away. The zoospores from germinating resting spores are similar in appearance, behavior, size, and structure to those from the evanescent zoosporangia. They have been observed coming to rest on incipient sporangia and intercalary swellings of the host and starting new infections. It is accordingly obvious that the resting spores described above are a part of the developmental cycle of *R. Cladochytrii*. Within a few days or weeks after germination, empty resting spores collapse and shrink.

ROZELLA ENDOCHYTRII.—This species parasitizes the sporangia of *Endochytrium operculatum* but does not cause hypertrophy or septation of the host. It was collected in April, 1941, from a water trough on the Karling estate near Austin, Texas, and has been kept in culture on its host at Columbia University up to the present time. It differs from *R. Cladochytrii* primarily in the shape, structure, and appearance of its zoospores, and secondarily because it does not cause hypertrophy and appears to be limited to a single host. Extensive inoculation experiments involving the same fungi used in studying *R. Cladochytrii* have been made, but all results obtained were negative. In this connection it may be further noted that in the oat leaves which harbored the parasitized sporangia of *E. operculatum* were numerous thalli and sporangia of *Rhizophlyctis petersenii*, but all of the latter were healthy and uninfected. In some portions of the leaves the infected sporangia of *E. operculatum* were almost surrounded by thalli of *R. petersenii*, thus affording ample opportunity for infection, but none of the latter thalli were attacked.

Because of the large size of the host sporangia, it has been impossible to follow the developmental phase of the parasite. Accordingly, less is known about this species than *R. Cladochytrii*. Infection of

young incipient sporangia has been seen but rarely (fig. 26), which may be partly due to the intramatrix habit of its host. In one instance a rounded zoospore was found attached to the main rhizoidal branch of *E. operculatum*, but germination and infection did not occur. Although it is possible that the zoospores may infect the rhizoids also, I have never found sporangia of the parasite in these organs. Since the host is an intramatrical saprophyte, it is difficult to conceive how the zoospores of *R. Endochytrii* get to it, unless they are capable of penetrating the substratum or unless parts of the host are directly exposed. Infected sporangia of *E. operculatum* have been found only in substrata which were decayed and undergoing degeneration. Under such conditions, *E. operculatum* may often be partially extramatrical and thus vulnerable to attack. Infection apparently occurs most abundantly when the host is young, but the fact that parasitized sporangia with long exit canals (fig. 29, 30) and fully developed opercula frequently occur suggests that full-grown sporangia also may be attacked. On the other hand, it is equally probable that the presence of the parasite does not inhibit development in the early stages of infection, so that the parasitized sporangium may often reach full development.

The symptoms of the disease and the internal appearance of infected sporangia are strikingly similar to those described in relation to *R. Cladochytrii*. As the parasite develops, the protoplasm becomes vacuolate, coarsely granular, with numerous irregular and globular bodies, and assumes the characteristic gleaming appearance (fig. 2). Further development involves fusion of the smaller vacuoles into one or two large central ones (fig. 28) and the accumulation of granular material in the center. The scalloped border of the central vacuole shown in figure 28 suggests that progressive cleavage has already begun, although the exit papilla is not evident. The latter may push through the wall of the host or use the opercular openings. In the latter event the operculum, if present, is pushed up (fig. 29), and the exit papilla projects out as a hyaline, dome-shaped plug. These may vary from one to four in number, and from 2 to 6 μ in height. The wall of the infected sporangium usually seems considerably thicker than that of healthy ones, which may be due to the addition of the parasite's surrounding membrane. Cleavage and delimitation of the zoospores appear to be the same as in *R. Cladochytrii*, although it has been impossible to follow the successive steps. Within sev-

Fig. 25-47.—Fig. 25-35. *Rozella Endochytrii*.—Fig. 25. Portion of a young thallus of *E. operculatum* with two attached zoospores of the parasite.—Fig. 26. Parasitized vacuolate sporangium of *E. operculatum* with coarsely-granular gleaming protoplasm.—Fig. 27. Later stage showing large scalloped central vacuole with numerous granules.—Fig. 28. Sporangium shortly before deliquescence of exit papilla.—Fig. 29. Emergence of the zoospores.—Fig. 30, 31. Enlarged view of pyriform and arched zoospores.—Fig. 32. Amoeboid zoospore.—Fig. 33. Large amoeboid zoospore which had four flagella.—Fig. 34. Encysted spore.—Fig. 35. Sporangium filled with slimy substance and extraneous bodies left behind by the parasite.—Fig. 36-47. *Rozella Rhizophlyctii*.—Fig. 36. Pyriform zoospore with a small globule and long posterior flagellum.—Fig. 37, 38. Amoeboid stages of same.—Fig. 39, 40. Bi- and triflagellate zoospores.—Fig. 41. Zoospore at close of swimming period.—Fig. 42. Multivacuolate stage of parasite with two exit papillae.—Fig. 43. Later stage with three large vacuoles.—Fig. 44. Parasite at the conclusion of cleavage.—Fig. 45. Emergence of the zoospores. A mass of spores are stuck together at the exit papilla.—Fig. 46. Mature resting spore.—Fig. 47. Empty resting spore which has apparently germinated.

eral minutes after cleavage has been completed, the zoospores begin to glide upon each other and upon the extraneous bodies among them. As in other species of *Rozella*, this movement increases in intensity until the zoospores are swirling in the sporangium and the exit papillae deliquesce. The deliquescence of the exit papillae, emergence of the zoospores, their initial behavior, and subsequent method of swimming are so similar to those described for *R. Cladochytrii* that it would be superfluous to describe them in detail again.

Their structure and appearance, however, are somewhat different. As is shown in figures 30, 31, and 32, they are usually obclavate to pyriform and sometimes arched. The anterior end tapers slightly, while the posterior is broader and rounded. Internally, they are optically homogeneous except for two and sometimes three denser globular areas. The anterior globule is usually smaller than the posterior one. These regions are not brilliant or particularly refringent, like the globules of most chytrids, but their refractive index is different from the surrounding protoplasm. They may be described as having a dull hyaline gleam, if such contradictory terms may be used. Consequently, when viewed under low magnifications, the darting zoospores give the impression of short rod-shaped, hyaline bacteria with two slightly more refractive endospores. When trapped in the sporangium or a tight place under the cover slip, they may become amoeboid (fig. 33), dragging the flagellum along behind. Most of them are $3.4\text{--}4\ \mu$ long \times $1.5\ \mu$ wide at the middle, and unflagellate, but occasional large bi-, tri-, and tetraflagellate zoospores also occur. Figure 34 shows a large amoeboid segment which possessed five flagella. Zoospores may also round up and encyst (fig. 35) at the edge of water drops and remain in this condition for many hours before degenerating.

The size and shape of the sporangia of *R. Endochytrii* vary according to those of the host cell. Sporangia up to $275\ \mu$ in diameter have been found. Since the zoospores are comparatively minute, the number produced in one sporangium is extremely great. Taking the diameter of an encysted spore (fig. 35) as an index of zoospore volume, the approximate number per sporangium may be computed. In the largest sporangia it obviously runs into the hundred thousands. It is accordingly not uncommon to find the entire mount teeming with darting zoospores when several large sporangia are dehiscing. As in other species of *Rozella*, zoospores which fail to find the exit pore may remain active for many hours before degenerating. Numerous granules, globular bodies, and slimy material are usually left behind in the sporangium (fig. 36) after the zoospores have emerged.

Resting spores have not been found in this species, although it has been kept in culture on its host for several months. Our knowledge of *R. Endochytrii* is accordingly incomplete, but the characteristic structure and appearance of its zoospores, nevertheless, set it apart from the other known members of *Rozella*.

zella. These characters together with the limited host range of the parasite, in my opinion, warrant the designation of this organism as a new species.

ROZELLA RHIZOPHLYCTII.—This species parasitizes the sporangia of *Rhizophlyctis petersenii* and is characterized by broadly pyriform zoospores with a comparatively long flagellum and spiny resting spores. Its host and substratum were collected from a ditch filled with rain water in a cow pasture on the Karling estate near Austin, Texas. This locality is doubtless the source of the parasite also, because no opportunity for contamination was presented in the subsequent handling and culturing of the host.

The shape and structure of the zoospore of this parasite differ from those of other species, and these differences coupled with a limited host range indicate that it is possibly a hitherto unknown member of *Rozella*. I am accordingly diagnosing it as a new species and proposing the name *R. Rhizophlyctii*. It is not improbable, however, that this species may be identical or related to the parasite which Miss Ward found in *Rhizophlyctis rosea*. She found only a few immature resting spores and referred them to an unidentified species of *Rozella*.

ROZELLA Rhizophlyctii *sp. nov.*—Sporangia solitary, filling host cell and conforming with the latter's size and shape, spherical, $20\text{--}110\ \mu$, oval, and irregular with 1 to 4 exit papillae which usually project out of the short necks of the host; wall of sporangium usually indistinguishable from that of the host cell. Zoospores hyaline, broadly pyriform, $2.5\text{--}3\ \mu \times 1.5\text{--}2\ \mu$, tapering slightly at the anterior end, with a minute globule near the posterior end; posteriorly unflagellate, rarely bi- and multiflagellate; flagellum $16\text{--}18\ \mu$ long; swirling in the sporangium before emerging; darting about rapidly in swimming, occasionally becoming amoeboid. Resting spores slightly yellow, oval and spherical, $14\text{--}18\ \mu$ in diam., with a large central vacuole and coarsely granular cytoplasm; wall spiny, $1.8\ \mu$ thick, spines $1.5\text{--}2\ \mu$ long; apparently transformed directly into a zoosporangium in germination and forming zoospores.

Parasitic in *Rhizophlyctis petersenii*, Austin, Texas, without causing apparent hypertrophy or septation of the host cells.

Fungus parasiticus; sporangia solitariis, longitudinem latitudinemque cellae matricialis in toto complentibus, spaeicis, $20\text{--}110\ \mu$, ovalibus, irregularibus, cum 1–4 papillis exeuntibus plerumque ex brevibus collis matricilibus se proicientibus; pariete sporangii ex cella matricali fere non discernendo. Zoosporis hyalinis, late pyriformibus, $2.5\text{--}3 \times 1.5\text{--}2\ \mu$, anteriore ex parte cacuminatis et minutissima gutta densissima in cytoplasmate praeditis; e posteriore unflagellatis, aliquando bi- et multiflagellatis; flagello $16\text{--}18\ \mu$ longo; se in sporangio torquentibus antequam emergunt; natantibus emicatum, raro amoeboides. Sporidis perdurantibus subflavis, ovalibus et sphaericis, $14\text{--}18\ \mu$ diametro, cum maxima gutta centrica et protoplasmate crasse granuloso; pariete spiculoso, spiculis $1.8\ \mu$ diametro et $1.5\text{--}2\ \mu$

longo; directa germinatione, ut videtur, in zoosporangium transformatis ut zoosporae formentur.

This species was believed to relate to a new small-spored species of *Rhizophlyctis* when first observed, but more intense study showed that the thalli at hand were those of *R. petersenii* infected with a foreign organism. As soon as this became evident, infection experiments involving the fungi used previously were made to determine the host range and pathogenicity of *R. Rhizophlyctii*. All attempts to infect other species failed, which indicates that this parasite is limited in host range.

As in the case of *R. Endochytrii*, it is difficult to determine with certainty whether or not this species causes hypertrophy of its host, because the normal host sporangia vary so greatly in size and shape. Although very large and irregular parasitized sporangia have often been found, it is not obvious that this large size is due to the presence of the parasite. The same is true in relation to observed irregularities in shape of infected sporangia. Internally, however, *R. Rhizophlyctii* has a conspicuous effect. The protoplasm of normal, healthy sporangia of *Rhizophlyctis petersenii* is usually salmon pink to light red in color, but in parasitized ones it is hyaline. As in the case of *R. Cladochytrii* on *Cladochytrium replicatum*, pigment formation and development is inhibited by the presence of the parasite. While it is true that in old cultures of *R. petersenii* growing on cellophane, sporangia may become almost hyaline, their appearance in contrast to parasitized ones is nonetheless different and recognizable.

The emergence, initial behavior, and motility of the zoospores of *R. Rhizophlyctii* are fundamentally similar to those of other *Rozella* species. In a few sporangia, however, masses of zoospores have been observed to emerge before they were completely separated. Such masses often remained together at the mouth of the exit papillae and obstructed the opening (fig. 46) or moved away as a unit shortly after emerging. In the former instance the zoospores pulled and twisted about, until the posterior flagellum was freed and stuck out at right angles to the group. Eventually the zoospores disengaged themselves and darted away. The behavior of these zoospores was very similar to that described by Fischer (1882) for *R. septigena*.

The zoospores of *R. Rhizophlyctii* are more broadly pyriform than in the previously described species, while the flagellum is on the average 3 to 4 μ longer. Most of them are uniflagellate, but occasional bi- and multiflagellate zoospores and irregular segments may occur (fig. 40, 41). Each spore includes a minute body which is not very refringent and appears almost back in transmitted light. One to several much smaller granules may also be present in the cytoplasm (fig. 37-40).

The method of infection, entrance of the parasite, appearance of infected sporangia (fig. 43, 44), delimitation, and maturation of the zoospores are much the same as those described for *R. Endochytrii*, so that the details need not be repeated again. Figure

45 shows a noteworthy sporangium in which the parasite apparently does not completely fill the host cell.

Only a few resting spores have been found to date, and all of these were spiny. They develop in the same manner as those of *R. Cladochytrii*, and no fundamental differences in the process have so far been observed. Likewise, their internal structure and appearance are essentially the same. Although no stages in germination and emergence of the zoospores have been seen, these processes are apparently similar to those of *R. Allomyces* and *R. Cladochytrii*. Three empty spores with exit pores were found in an old culture, which suggests that the resting spores of this species also function directly as zoosporangia and form zoospores within.

Discussion.—The three species described in this paper appear to belong in the so-called "sporangium-group" of *Rozella* and have posteriorly uniflagellate zoospores. The addition of these species brings the number of members in this group to twelve or possibly thirteen, while only two certain and two very doubtful species belong in the "septigena-group." The discovery of three additional species with posteriorly uniflagellate motile cells is further proof that *Rozella* is characterized by zoospores of this type instead of biflagellate heterocont ones as Fischer maintained. It is thus obvious, as Sparrow has already pointed out, that *Rozella* should be retained in the original sense of Cornu. In that event, *Pleolpidium* becomes a synonym of *Rozella*. Whether or not Cornu's *R. septigena* and *R. Allomyces* Foust should be included in this genus depends on the interpretation of *Rozella* and remains to be determined from careful cytological studies. If, as is now generally believed, each infection gives rise to a single sporangium in the non-septigenous group, whereas in the "septigena-group" a plasmodium is formed which segments into a number of portions and gives rise to several sporangia, these differences in type of development may be significant enough to justify the creation of a new genus for the septigenous species. Conclusive evidence of such differences has not yet been presented, and it is accordingly wiser to retain the two groups within the same genus for the time being.

Pleolpidium inflatum Butler and the parasite described by Miss Waterhouse in *Phytophthora*, however, cannot be included in *Rozella*, because both species have biflagellate zoospores. Otherwise they appear to resemble *Rozella* species of the "sporangium-group," although they are not yet fully known. No septation of the hypertrophied host cells is produced, and each infection seems to give rise to a single sporangium. The structure of the zoospores, nevertheless, excludes these species from *Rozella*, and it is obvious that they should be placed in a separate genus. Since they do not fit into any of the known genera which are characterized by zoospores of this type, a new genus, *Rozellopsis*, is proposed for these species.

The disposition of Fischer's biflagellate, heterocont *R. septigena* and *R. simulans*, however, remains unsettled. Fischer claimed that his *R. septigena* is the same as Cornu's species but occurs only on species of *Saprolegnia*, while *R. simulans* is limited to *Achlya*. The species are otherwise alike. It is not improbable that Fischer may have been incorrect about the number, place of insertion, and relative lengths of the flagella in *R. septigena* Cornu, or he may have attached too much significance to the occasional large, abnormal biflagellate zoospores and regarded them as typical of this species. Such zoospores were previously observed by Cornu and later by Sorokin (1883), but they regarded them as abnormal. On the other hand, it is equally probable that Fischer actually had at hand a *Saprolegnia*-inhabiting, septigenous species with biflagellate, heterocont zoospore which he took to be Cornu's *R. septigena*. Tokunaga's confirmation of biflagellate zoospores in *R. simulans* supports the probability of the existence of septigenous species with zoospores of this type. It is also not improbable that Fischer's *R. septigena* and *R. simulans* may be identical or that the latter species is a physiological variety of the former. Subsequent workers have accepted Fischer's inoculation results without much question. Maurizio (1895), Minden, and Tokunaga subsequently reported this species in *Achlya* sp., *A. racemosa*, and *A. flagellata* from Switzerland, Germany, Italy, and Japan, respectively, but none of them tested its pathogenicity to species of *Saprolegnia*. The resting spores which Minden figured in the short hypertrophied side branches of *A. racemosa* are strikingly similar to those of *R. septigena*, which further suggests that the two species may be identical or closely related. However, until further studies have been made, their identity and relationship will remain doubtful.

Whether or not *R. septigena* Fischer and *R. simulans* may be included with Butler's and Miss Waterhouse's species in the new genus *Rozellopsis* is not

certain, because resting spores have not been observed in the latter two species. In the interests of clarity and convenience, however, it may be worth while to include them in this genus for the time being. *Rozellopsis* accordingly becomes a parallel genus with *Rozella* by including both "sporangium" and "septigenous" biflagellate, heterocont species. Under this interpretation, *Rozellopsis* includes four species: *R. inflatum* (Butler) comb. nov., *R. waterhousei* sp. nov., *R. septigena* (Fischer) comb. nov., and *R. simulans* (Fischer) comb. nov.

SUMMARY

Parasitism among chytrids appears to be comparatively rare; only nine cases have been reported up to the present time. Of the nine species known, none attacks hosts which belong to the same genus as the parasite, and only one occurs on a host of the same family. Five of the nine parasites belong to the genus *Rozella* and cause marked local hypertrophy or septation of the host cell, or both.

Infection experiments show that *R. Cladochytrii* is ubiquitous in host range. It parasitizes three species each of *Cladochytrium* and *Nowakowskiella*, but does not attack monocentric rhizidaceous chytrids or filamentous Oomycetes. *Rozella Endochytrii* and *R. Rhizophlyctis* sp. nov. appear to be limited to *Endochytrium operculatum* and *Rhizophlyctis petersenii*, respectively.

The results of this study show that the zoospores of *Rozella* are predominantly posteriorly uniflagellate and thus support the view that this genus should be retained in the original sense of Cornu. *Pleolpidium* is accordingly reduced to the status of a synonym of *Rozella*, and a new genus, *Rozellopsis*, is proposed for the biflagellate, heterocont, *Rozella*-like species reported previously in the literature.

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A CYTOLOGICAL STUDY OF THE CAROTENE IN THE ROOT OF DAUCUS CAROTA UNDER VARIOUS EXPERIMENTAL TREATMENTS¹

T. E. Weier

SO FAR AS I am aware, Arthur Meyer's description (1883) of the carotene bodies in the carrot root is still the most complete and detailed report on the subject. In cells close to the cambium he describes very small reddish bodies which enclose starch grains. Farther from the cambium these bodies and the enclosed starch grains enlarge, the bodies still maintaining their reddish or orange color. Eventually the starch is dissolved. In cells containing but very little starch he found either: (1) small yellowish granules which are recognized as starch-free leucoplasts, or (2) many such leucoplasts grouped together in one large mass, or (3) thin-walled vesicles which can be interpreted as having originated through the dissolution of the enlarged starch grains, or (4) many characteristic tubes and needles of carotene. In the outer portions of healthy and older carrots he found very many well-formed rectangular or rhomboidal bodies. Meyer also noted rows of several starch grains enclosed in a narrow ribbon of carotene and other cases in which only a single starch grain was embedded in the pigment. In some instances only a portion of pigment body was occupied by the starch grain, while in others the starch grain was attached only at one end of the chromoplast.

Meyer believed, though without a great deal of evidence, that the stroma of the plastid was gradually absorbed as the cells aged. While this process was going on, there occurred an accumulation of the pigment around the starch grain resulting in the formation of a tube of pigment. Finally the starch grain also dissolved, the dissolution being most rapid where it came in contact with the pigment. When the starch had completely disappeared the tube of pigment stretched, forming the typical carotene crystal of the carrot root.

Meyer noted, in addition to these bodies, smaller brownish granules which were clumped together in large masses, and which he interpreted as degradation products of the carotene crystals. Within the xylem region he noted very small reddish and yellowish-green pigmented bodies.

In cold alcohol the crystals of carotene in the outer portion of the older carrots dissolved very slowly. When hot alcohol was used they disappeared rapidly

and left no residue. From this experiment Meyer concluded that the pigment bodies were composed of pure pigment not containing any stroma or *Farbstoffträger*. Further experiments led him to believe that the rods and plates of pigment were pure carotene. He believed that, as long as the pigment was in young active cells, it was associated with a cytoplasmic stroma. In older cells this cytoplasmic material was withdrawn. So long as the carotene is mixed with other substances, the crystallization force results in tubes and spirals; later, when the carotene becomes entirely free from associated material, real crystals are formed.

Guilliermond's (1933) description of the carotene in carrot is somewhat similar. He believes that the carotene and starch form within mitochondria, that the starch is resorbed, and that sometime before the pigment becomes pure and crystalline the mitochondrial substrate is withdrawn, leaving the crystal of carotene free in the cytoplasm.

According to Strain (1938) the leaf xanthophylls are quite probably associated with cellular proteins and lipoids. The evidence for this is based upon the relative absorption properties of the pigments in living cells and in test tubes, the action of heat and enzymes on leaves, the extraction and separation of the pigments in green and etiolated leaves, and the reactive properties of the pigments in combination within the cell and of these same pigments after the combination has been disrupted by the action of chemical reagents, enzymes or heat.

The present study reports on the microscopic appearance of the carotene in the cells of the carrot root. The pigment is present in discrete crystal-like bodies, associated with starch grains in a complex, resembling plastids, and in irregular small patches of cytoplasm. The behavior of the pigment following changes in the cytoplasm, such as liquefaction, coagulation and desiccation, is dependent upon its association with the cytoplasm.

METHODS.—Field grown carrots were used exclusively. These were grown in the field throughout the year in Davis. While this assured a constant supply, it introduced a seasonal variation in carotene and starch content which, when combined with possible varietal differences, introduced factors which cannot

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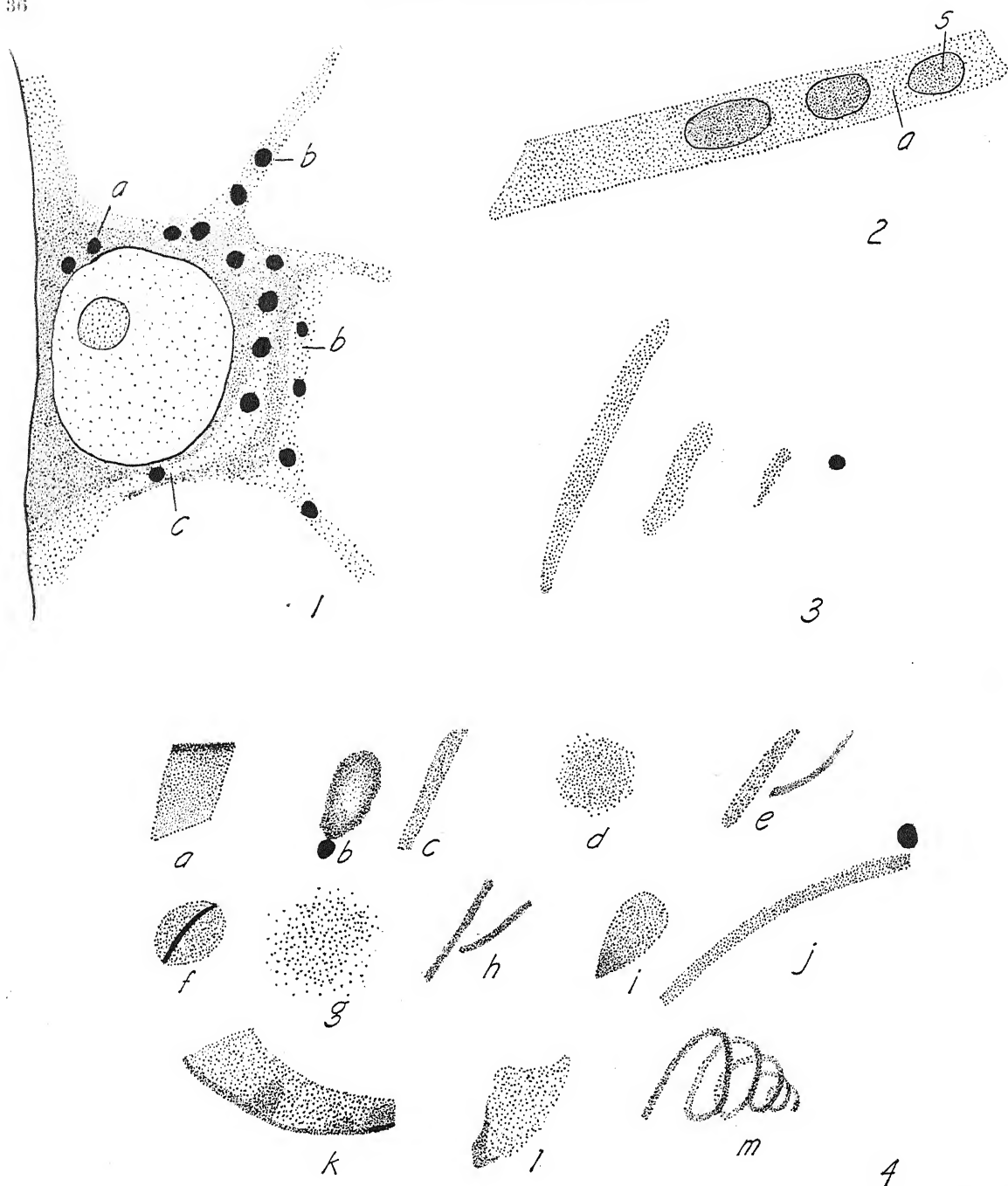


Fig. 1-4.—Fig. 1. Developing cell showing yellowing cytoplasm heavily stippled surrounding nucleus. Granules (b) move in colorless streaming cytoplasm (c), and among stationary granules (a).—Fig. 2. A carotene ribbon (a) in which starch (s) is embedded.—Fig. 3. Steps in the dissolution of a narrow ribbon of carotene from a section mounted in alcohol. A small colorless granule remains.—Fig. 4. Various types of pigment bodies found between the cambium and the first vessel.

be successfully studied by cytological techniques alone.

Carotene is easily destroyed within the cells. The usual cytological techniques either dissolve or decompose it. It is preserved quite well by weak osmic acid and may be observed in good condition in living cells,

for which reason free-hand sections were used exclusively in this study. This treatment, obviously, does not permit of a thorough investigation of all cytoplasmic structures within the cell, nor of the development of the carotene and of other bodies in the meristematic cells.

Free-hand sections made from freshly gathered carrots were examined in M/40 phosphate buffers with a pH of 7.0. No observations were carried out on sections for longer than four hours. During this time cyclosis was active, and no signs of cellular degeneration in uninjured cells were apparent.

The solvent action of pyridene, various strengths of ethyl alcohol, methyl alcohol, petroleum ether, chloroform, the action of sodium oleate, of formaldehyde, of phenol and HCN were observed directly on the living material. The free-hand sections were first mounted in the phosphate buffer, the cytoplasmic activity noted and the reagent drawn under the cover glass. Its action was then observed. The effects of heat and of desiccation on the cytoplasm and pigment were studied by drying the sections at temperatures ranging from 4°C. to over 200°C.

Observations were carried out in both direct and dark field illumination. In direct light Zeiss apochromatic objectives 120×, N. A. 1.3 and 90× N. A. 1.3 were used; for dark field work the 60× N. A. 1.0 apochromatic objective supplied with a diaphragm was used; the dark background was supplied by a Zeiss cardioid condenser.

THE MACROSCOPIC REGIONS OF THE CARROT ROOT.²
—The ontogeny and structure of the fleshy storage organ (root and hypocotyl) of *Daucus carota* have been recently considered by Esau (1940). The following description of the macroscopic structure of the carrot root utilizes the information contained in the above reference and adds some new observations on fresh sections.

The central portion of the root, the core, is composed of the xylem; the peripheral portion is mostly phloem. A narrow layer of pericycle and periderm cells occurs on the periphery of the phloem. Both the xylem and the phloem are highly parenchymatous and are separated from each other by a narrow translucent band of cells, the cambium.

In fresh sections of the carrot root, translucent and opaque areas are combined into a characteristic pattern. The major opacities are formed by several structures, the rays, the parenchyma accompanying the secondary roots, and by the secretory tissue. The rays and the secondary roots form the radiating sheets of tissue, and the oil ducts with their secretory cells appear in concentric layers crossing the rays. The opacity of these regions results from the presence of numerous large intercellular spaces. The parenchyma of the pericycle and of the vertical system of the xylem and phloem is comparatively compact and appears translucent. The vessels and the sieve tubes are imbedded in the translucent areas, although the vessels themselves are recognizable as very small opaque spots. The opaque rays in the phloem extend almost to the periphery of the organ and give an indication of the amount of tissue contributed by the phloem. The narrow layer beyond the rays is composed of pericyclic cells and of periderm of pericyclic origin.

² The writer acknowledges the assistance of K. Esau in the preparation of this part.

THE MICROSCOPIC APPEARANCE OF THE CAROTENE. —Carotene in cells differentiating from cambium.—

The greatest concentration of the carotene occurs in the parenchyma cells of the phloem and the pericycle. There is no visible difference between the carotene content of the opaque rays or of the more translucent areas described above. Both regions seem to have the same amounts and types of carotene crystals. A much smaller amount of pigment forms in the xylem. Its formation here seems to be generally more rapid than in the phloem. Xylem cells close to cambium contain large numbers of small bodies of carotene, while in the phloem well-developed carotene crystals appear usually only in the more mature tissue. This condition renders the phloem and pericycle more favorable for the study of the pigment and the great majority of the observations were carried out in these tissues.

In differentiating cells of carrots collected during the summer months, the pigment appears in two forms: as definite small crystals and as an amorphous mass tingeing the cytoplasm.

Relatively large clumps of cytoplasm are frequently observed to have a yellowish tinge. While it seems reasonable to assume that this color is due to carotene, it is well to bear in mind that a delicate shade of yellow in the cytoplasm is the only evidence. Treatment with sulphuric acid destroys the cell without giving any direct evidence one way or the other. Such patches of cytoplasm tinged with color have been noted many times, both in cells exhibiting active cyclosis and in those devoid of it.

A portion of one cell with actively streaming cytoplasm is shown in figure 1. The relatively large mass of cytoplasm around the nucleus was colored. Stationary granules (a) were noted close to the nucleus, while along the periphery of the tinged mass of cytoplasm the rapidly streaming, colorless cytoplasm carried other granules (b). There was, in addition, a slow movement of granules in a colorless channel (c) through the tinged cytoplasm.

A similar situation was observed in an adjacent cell. Granules moving in the cytoplasmic strand along the periphery of a stationary tinged mass of cytoplasm were shunted aside to become enmeshed in the motionless cytoplasm. Small crystals were also present. The tinged mass of cytoplasm gradually changed shape, the small crystals and granules constantly assuming new positions with reference to each other. In doing so they moved slowly through the colored mass of cytoplasm.

In another cell showing active cyclosis the tinged motionless mass of cytoplasm was confined to one end of the cell. The strand, moving rapidly along one edge of this mass, gradually eroded it away.

Occasionally one obtains a good three dimensional view in which the side wall of the cell is at a slight angle with the rays of light passing through the preparation. In one such case the cytoplasm adjacent to this wall appeared as a gently rolling field. Much of it was colorless, but one region containing

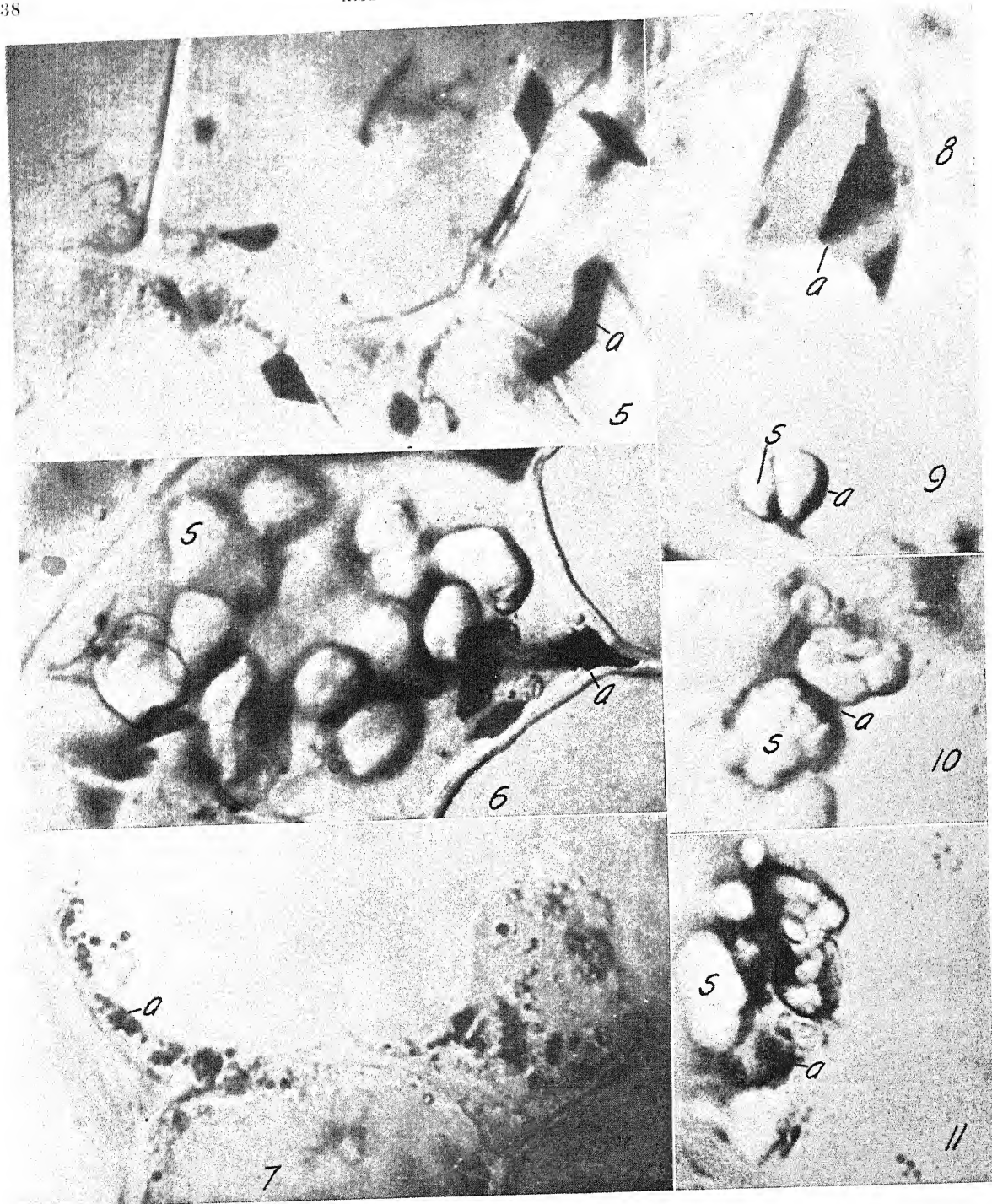


Fig. 5-11.—Fig. 5. Various types of crystals (a) found in an old phloem cell; no starch present.—Fig. 6. Carotene crystals (a) and starch grains (s) in a phloem cell.—Fig. 7. Upon drying, the pigment (a) forms yellowish droplets around the periphery of the cell.—Fig. 8. A large flake of carotene in older phloem cell.—Fig. 9 and 10. Carotene (a) associated with starch grains (s).—Fig. 11. Large clump of starch grains (s) associated with carotene (a).

several small starch grains was lightly tinged with pigment.

Color was never observed in the streaming strands, even when yellowish motionless masses were being eroded away by rapidly moving strands. This is

probably the case because the pigment is so dilute in the strands that it cannot be observed.

Reports of cytoplasm tinged with chlorophyll persist in the literature. They were especially frequent in the older literature. Modern conceptions of the na-

ture of the chloroplast have led to the interpretation that the presence of colored cytoplasm in the cell was due to injury (Weier, 1938). Bold (1940) has, however, recently described a situation in living uncut moss sporophytes of such a nature that makes this interpretation impossible. Evidence is accumulating which leads to the conclusion that in certain, probably exceptional circumstances, the leaf pigments may permeate the general cytoplasm of the cell.

The pigment also occurs in differentiating cells as small crystals similar in appearance to those found in the mature cells. They do not appear to be associated with any specialized region of cytoplasm. In dark field illumination they are brilliantly red, blue and green. This color is not due to fluorescence, as it disappears when the sections are illuminated with those wave lengths of light which are absorbed by the carotene. The small crystals may be traced down to minute points of light and seem to grade down to the limit of visibility. The larger bodies are distinctly carotene crystals. There is some question as to whether the smaller ones are. They resemble the larger bodies in their defraction of the oblique light of the dark field illuminator, in their solubility in alcohol and in their brilliant double refraction between crossed nicols. However, in direct illumination there is no suggestion that the small particles of pigment grade down to the limit of visibility, although such small bodies of carotene might be present, yet quite invisible, with direct illumination.

All of these bodies with intense dark field illumination have a clear outline of light surrounding them. Beck (1938) describes this phenomenon and points out that it is generally caused by the first and most brilliant diffraction ring in the image of each point of the object. It is not to be interpreted as a structural part of the object but is due entirely to diffraction.

In direct illumination the number of different shapes assumed by the pigment in differentiating cells is very large. Figure 4 shows a series of such bodies in the order in which they appeared from the cambium to the first vessel. In the phloem such a seriation would be derived from many more cells. In these xylem cells there are several irregular faintly tinged (d, g, l) masses of cytoplasm, small needle-shaped bodies (e, h), flakes (a, k), ribbons (j), and a spiral (m). None of them gave any evidence of being associated with a specialized mass of cytoplasm.

In osmic acid preparations the carotene bodies are visible in both direct and dark field illumination. Although definitely embedded in the cytoplasm, no differentiated mass of cytoplasm was observed to be associated with them. In some cases blackened granules were in contact with the pigment bodies. Since both pigment crystals and granules are present in the rapidly streaming cytoplasm, a certain number of such contacts should be expected.

While I have emphasized the apparent lack of association of the pigment bodies with any organized cytoplasm or plastid, conclusive evidence has not

been obtained that these carotene bodies did not originate in association with some plastid substance.

Carotene in the cells of the phloem and pericycle.—The development of the carotene crystals in the phloem and pericycle is puzzling, and only a brief description of the appearance of the pigment in the various cells will be given, without any attempt to outline the steps in the formation of the spirals and needles found in the older cells. There are two technical difficulties which make for this confusion. First, a clear-cut seriation of events is lacking. Many types of pigment bodies are found in the same cell, although there may be general differences in the numbers and types of bodies in different regions of the root. Secondly, field grown carrots of different varieties and sizes collected at different times of the year vary in starch and carotene content.

The young carrots examined in the laboratory usually contained but little starch, while the carotene was present in a confusing array of forms. Older, though still immature, field grown carrots examined during the summer months presented the simplest picture. In these carrots three regions outside of the cambium may be distinguished by their starch and pigment content. The first, a differentiating region, is composed of cells which contain little starch and pigment. The second region comprises a varying extent of mature cells containing numerous large starch grains and considerable pigment. The plastid component associated with the starch grains has been stretched so as to be invisible either in living or fixed and stained sections. These grains are usually clumped together (fig. 11). In cells showing active cytoplasmic streaming they move in the cytoplasmic strands. Sometimes the starch grains of a given clump will separate from each other; at other times two or more clumps will join to form a single large mass. Single starch grains may leave the larger clump to move around the cell alone. In the few instances in which observation was continued until movement ceased, the starch grains came to rest in that region of the cell receiving the least amount of light.

The pigment may be present in these cells either as isolated well-formed crystals (fig. 6 and 8), or it may be associated with clumps of starch grains (fig. 9, 10, and 11). In the latter case the whole mass of starch grains (or various portions of the mass) is orange in color. The darker regions (a) in figures 9, 10, and 11 are pigment. This effect may be brought about either by crystals or by carotene in close association with starch. The starch grains are transparent and refract light passing through them, so that crystals tightly massed with starch grains are not visible as such. They may be detected in cells under conditions of cytoplasmic streaming, active enough to separate the starch grains and to free the crystals from the mass. The association of starch and carotene may be recognized by the presence of the pigment between two or more starch grains (fig. 9a and 10a), by its location on starch grains or by the failure of crystals to appear when masses of

starch grains revolve or separate without disclosing crystals. In many cells not showing cyclosis it is impossible to decide whether or not the coloring of the starch mass is due to crystals of carotene or to the association of the pigment with the starch grains.

The third region is differentiated by the lack of starch (fig. 5). It is probably composed mostly of pericycle cells with some of the older phloem cells. This region may constitute the outer half of the carrot, although it usually is much smaller, being confined to from twenty to a hundred cells just inside of the periderm. The pigment is found in most carrots in two distinct colors, red and orange. The orange usually predominates and some carrots may have only orange crystals. It is also found in many diverse forms. Flakes, needles, spirals, long ribbons, short ribbons, narrow ribbons, and broad ribbons are all to be seen in both colors (fig. 5 and 6). One type of body is particularly interesting. It is a long ribbon (fig. 2a) in which one to several small starch grains (s) are embedded. This seems to be one stage in the dissolution of starch and of the subsequent freeing of the carotene and was so interpreted by Meyer. Lack of intermediate stages does not permit a definite conclusion.

These crystals resemble those of differentiating cells in their appearance in dark field illumination and between crossed nicols.

THE EFFECT OF ETHYL ALCOHOL ON THE CAROTENE IN THE DIFFERENT REGIONS OF THE CARROT ROOT.—*Differentiating cells.*—The small size of the pigment bodies in these cells makes study difficult in this region. Cold alcohol dissolves the pigment slowly; no droplets or other type of body were observed to form. It is not possible to say whether or not residue or stroma is left behind after the pigment has been completely dissolved.

Mature cells containing large amounts of starch.—Alcohols of a dilution up to 60 per cent usually have little effect on the pigment in these cells. However, when stronger alcohol is used, the pigment disappears, and olive green droplets appear within the cells (similar to fig. 7). There is some variation in the reaction of these cells to alcohol. Sometimes many droplets form which are relatively insoluble in alcohol. In other instances few droplets form which may dissolve in alcohol. There seems to be some correlation between the number of droplets formed and the condition of the pigment in the cells. More droplets are formed when the pigment is closely associated with the starch grains than when crystals are present. It is not possible to follow the minute changes that are taking place within the cells as the pigment disappears and the droplets form. It is probable that a lipophaneroze (Beauverie, 1926) occurs: the cytoplasm is broken down by the action of the alcohol, releasing lipoids which are present in sufficient quantity to dissolve all of the pigment present. The lipoid thus released forms droplets in which the pigment dissolves, the solution finally being extruded from the mass of starch grain-cytoplasm-pigment complex. Droplets forming in cells with few or

no crystals are slowly if at all soluble in alcohol. They do disappear after several days, but this is quite as likely to be a chemical disintegration as dissolution. The droplets dissolve instantly in chloroform.

Mature cells devoid of starch.—While I have divided the carrot root into three regions for convenience, it must be borne in mind that these regions are not distinctly segregated, nor do they correspond with the gross morphological regions describe above. In general the translucent redder areas contain fewer starch grains, although this is at best a broad generalization.

Dilute alcohol has little or no effect on the carotene bodies in these older cells. Neither 60 nor 80 per cent alcohol, which induce the formation of droplets in starch-containing cells, have any great effect on the carotene crystals.

Ribbons of carotene were occasionally found to fray out at the ends. Less frequently elongated lines or fibers could be observed within the ribbon. This suggests that the ribbon has a parallel alignment of micelles as has been suggested for the chloroplast (Menke and Koydl, 1939) and is in agreement with the doubly refractive nature of the carotene bodies. These various changes in the appearance of the carotene crystals could not be duplicated at will. They occurred infrequently and unexpectedly during the study.

As already mentioned, the pigment is present in most cells in two colors, red and orange. Since in dilute solutions carotene is orange in color and changes to red abruptly at a relatively high concentration, it was thought at first that the color of the pigment was a function of the dilution of the pigment. The ribbons, flakes, and spirals were supposed to be more dilute, that is associated with some lipoidal material (see below), while the needles, being more crystalline, were thought to be pure pigment and should therefore appear red. While this hypothesis to explain the color differences in the pigment bodies held for a number of observations, carrots were obtained late in the fall which contained pigment bodies of all shapes in all colors. There were red flakes and orange needles. When 95 per cent alcohol is drawn over sections of carrots containing bodies of both colors, the orange colored crystals invariably disappear first. I have observed them dissolve completely in one hour, while three hours later the section still contained numerous red crystals.

In 95 per cent alcohol the carotene bodies dissolve slowly. There was no typical pattern in this dissolution. In some cases it was a simple wearing away of the body; in others the ribbon would become vacuolate, the vacuoles coalescing until all of the pigment had disappeared.

In the case of the ribbons or flakes a small colorless residue remained behind. The following examples are taken from my notes:

1. An elongated flattened needle-shaped body was selected for observation in direct illumination.

- a. Ninety-five per cent alcohol was drawn under the cover slip.
- b. Darker granules, surrounded by a clear space appeared within the pigment. These suggested bordered bits of pine tracheids.
- c. These holes enlarged and coalesced, while the borders of the ribbon remained intact and distinct.
- d. A chain or irregular series of isolated colorless granules remained behind as a residue.

Turning to dark field illumination two other elongated ribbons were watched. Vacuoles did not appear in these bodies; they gradually became smaller and smaller. One (fig. 3) was reduced in size as shown. The small granule remaining as residue was quite indistinguishable from any of the other cytoplasmic granules.

The second body broke up into two granules and a group of several small granules very close together. The two small granules were a delicate green in color and they eventually disappeared. The group of granules coalesced to form one colorless body which did not dissolve in the alcohol.

The action of chloroform on the residue was studied. A large flake of carotene was selected in one of these observations. The pigment was dissolved leaving a small colorless flake which was washed with 95 per cent alcohol for forty-five minutes without further change. Chloroform was then drawn under the slip. The colorless flake dissolved immediately leaving no visible trace.

In another instance two long red needles were observed as the alcohol dissolved away the pigment. Here the residue remaining behind retained approximately the shape of the original crystal. When chloroform was drawn under the cover slip, the residues dissolved immediately.

In one case about one quarter of a needle of carotene dissolved completely soon after the alcohol came in contact with it. The remaining three-quarters did not dissolve, although continually washed with 95 per cent alcohol for some time.

On the other hand needles and other crystal-like bodies usually dissolved completely, leaving no visible residue.

THE EFFECT OF DESICCATION UPON THE CAROTENE.—Strain's observations (1938) of the rate of oxidation of the carotinoid pigments led him to the opinion that they were associated or in actual combination with fats or proteins within the cell. That a residue remains when the crystals of carotene are dissolved in alcohol within the cells of the carrot root is definite evidence that this is the case. It was thought that further information might be obtained on this point through a study of the melting points of the carotene within the cells. Such results were, however, unobtainable as the carotene bodies were transformed into greenish globules of pigment upon simply drying the section.

Fresh sections were dried at 100°C., 30°C., 22°C., and 4°C. In all cases the typical pigment bodies dis-

appeared with the formation of yellowish green droplets (fig. 7). These droplets closely resembled those formed in the starch-containing cells when they were placed in alcohol, except that upon drying the droplets appeared evenly distributed throughout all cells of the root.

Sections were dried in a vacuum over calcium chloride for nineteen hours. Droplets appeared under these conditions resembling those that form when sections are dried in air. While not constituting proof that oxidative changes are not responsible for the disorganization, it would lend evidence for that assumption.

Sections were fixed by placing them in a dilute solution of osmium tetroxide for four days. They were washed and examined. The carotene bodies were well preserved. Drying had no noticeable effect upon them. Sections were heated to a temperature of 205°C., which is considerably above the melting point of pure carotene, without apparently affecting the pigment bodies. The action of the heat on the pigment cannot, of course, be followed directly microscopically. The sections were heated and then mounted on a slide for observation. It is possible that the pigment melted and crystallized again in cooling. However, after osmium fixation, the pigment bodies are not transformed into droplets upon drying or heating the sections.

I think that the most logical explanation of these observations is that a disorganization of the cytoplasmic structure occurs upon drying. Lipoids are thus released which in turn dissolve the carotene. Dr. Strain informs me that there is sufficient fatty material within the carrot root to dissolve all of the pigment present. When the sections are treated with osmic acid, the cellular lipoids and proteins are hardened, and subsequently the release of the lipoids does not take place. Carotene is not blackened by the osmium; this would indicate that lipid material associated with it is probably completely saturated.

Petroleum ether.—The carrot selected for treatment with this reagent contained many crystals of carotene in outer cells, while the cells closer to the cambium were well supplied with masses of starch grains and associated pigment. When petroleum ether is drawn over sections mounted in water, no change takes place even after 45 minutes.

Sections were mounted dry, brought into focus on the microscope stage in the shortest possible time and petroleum ether drawn over the sections. There is immediate and complete disorganization. The crystal-containing cells in the outer phloem resemble those having received heat treatment (fig. 7); most of the crystals have disappeared, only a large number of yellowish droplets being visible within the cell.

Additional evidence that the pigment does not go directly into solution in the petroleum ether, but that lipophanerosis occurs and that the solvent is in some manner associated with the cytoplasm, was obtained in another observation. A rather large irregular mass of yellow cytoplasm in which a large ribbon crystal

was partially embedded was watched for some time. Since the pigment bodies had been observed to leave behind a yellow residue when disintegrating after petroleum ether treatment, the yellow color of this cytoplasm must have been due to the carotene. This yellow pigment gradually disappeared, whether through solution in the petroleum ether or through disintegration it is not possible to say. After the cytoplasmic mass had become quite colorless, the red ribbon of carotene gradually became diffuse and smaller, while the colorless mass of cytoplasm again became yellow. There can be no doubt but that the carotene stains the cytoplasm. The mass of cytoplasm remained colored for about ten minutes. After this time the pigment had been completely washed from this cell. About ninety minutes of continued washing with petroleum ether was required for this change. Other cells still contained both crystals of pigment and yellow droplets.

When chloroform replaced the petroleum ether in the sections, large yellowish masses appeared in the cells containing starch, while many yellowish droplets close to the cell wall were noted in the outer phloem cells. The yellow substance is either insoluble or only slightly so in chloroform.

The action of two crystals of pigment under the influence of petroleum ether was checked. In one case the needle of carotene swelled considerably and the pigment gradually disappeared, this time without the coincident appearance of yellow color within the cell. An irregular colorless mass about the size of swollen crystals remained.

In another cell a ribbon was observed to disappear, leaving in its stead a number of irregular yellow bodies.

Methyl alcohol.—Carotene is not soluble in methyl alcohol. When it is drawn over a section of carrot root, the pigment disappears very slowly with the subsequent formation of yellow droplets. Both yellow and red crystals were present in the cells under observation. No difference in their action toward methyl alcohol was noted.

THE ACTION OF FORMOL, PHENOL, AND HYDROGEN CYANIDE ON CAROTENE.—The action of these three substances on the carotene crystals was studied. Formol was chosen because it coagulates the proteins and probably hardens some of the fatty materials. Commercial formaldehyde as well as a two per cent solution were used. Phenol and HCN both kill by liquefying the cytoplasm, HCN, in addition, inhibiting the oxidative changes which take place upon the death of the cell. The concentration of the phenol was two per cent.

There was no change in the appearance of carotene crystals after three days' immersion in the formols, two per cent phenol, and the HCN. When alcohol was drawn over sections which had been previously treated for twenty-four hours with these solutions, the pigment was found to dissolve rather more slowly than from fresh sections. After treatment with two per cent formol the red crystals were very resistant to the action of alcohol. In the phenol treat-

ed material, alcohol caused the formation of greenish droplets which soon disappeared. Red crystals were noted after the sections had been immersed in alcohol for twenty-four hours. Alcohol brings about the dissolution of the pigment bodies in the HCN treated material only very slowly.

When chloroform was drawn over the treated sections mounted in alcohol, the following results were obtained: in 2 per cent phenol, the crystals disappeared without the formation of any droplets; with HCN, brilliant yellow droplets formed, the intensity of the color probably being due to the inhibition of the oxidative changes which normally occur upon death of the cytoplasm; 1 per cent formol caused the formation of greenish droplets. The reaction is fundamentally one between the cellular constituents and the solvents, for these droplets will form even after the sections have been in alcohol for sufficient time to dissolve out all of the pigment. In some sections containing pigment, colorless globules are first formed, the pigment subsequently passing into solution in them. They form rapidly from various points within the cell and enlarge and coalesce until one large globule fills the cell cavity.

There is considerable difference to be noted here from the action of chloroform and alcohol on fresh sections. In the latter, alcohol induces the formation of droplets only in the starch-containing cells where the carotene is not visible in the form of discrete bodies. When crystals of pigment are present, the alcohol dissolves them gradually without the formation of droplets. Chloroform added to these sections results in the complete dissolution of the pigment, leaving the cell washed clear of any color.

When formol, phenol and HCN treated sections are washed and dried, greenish droplets are apparently formed in all cases. In the formol sections, however, this granular stage did not last very long; the sections became faintly colored throughout and soon lost the pigment. In the phenol treated sections the droplet formation was very slow, as crystals were noted after four days of drying. In HCN the droplets formed readily upon drying; they were yellow and eventually disappeared.

ON THE NATURE OF THE CAROTENE BODIES.—There is some doubt as to whether the carotene bodies should be termed chromoplasts, crystals or neither. The pigment is believed to be present either in a crystalline form or in association with some sort of cytoplasmic matrix, *i.e.*, a plastid. Some of the shapes are distinctly crystalline, particularly the long needles which are so frequently encountered. The curved bodies and the long cone-shaped spirals have no counterpart among true crystals. Meyer (1883) considered the needles to be crystalline, and held that, when the pigment was present in the shape of flakes, ribbons or spirals, it was associated with a cytoplasmic matrix. Guilliermond (1933) considered all of these distinct pigment bodies to be crystals.

Diluted pigment is orange in color. At a higher concentration it turns a deep red. If Meyer's interpretation is correct, the flakes, ribbons, etc., which

he supposed to be associated with cytoplasm, should be orange, while the needles should be deep red. This is not the case, for all types of pigment bodies may be either red or orange. Furthermore, according to both Meyer and Guillaumond, when starch and pigment are associated, a cytoplasmic matrix is still present. Yet I have, not infrequently, observed well-formed needles which are supposed to be pure pigment associated with starch.

There can be no doubt that, when large amounts of starch are present, the association between starch, pigment, and cytoplasm is not similar to that occurring when the pigment bodies predominate. The experiments with alcohol indicate this. The question arises, however, as to whether one is to consider this starch-pigment-cytoplasm complex as a typical plastid. Morphologically it does not resemble a plastid. It may be a mass of plastids or some other specialized grouping or arrangement. It is true that the chromoplasts in other cells, such as those of *Gaillardia* hairs, have an amorphous shape; yet one has no difficulty here in recognizing the essential plastid nature.

The experiments with alcohol have already brought out differences between the reactions of the pigment bodies in the carrot and typical chloroplasts. It seemed worth while to treat the material with other reagents toward which plastids have definite reactions.

Sodium oleate.—When typical plastids are treated with a dilute solution of sodium oleate, very definite and characteristic myelin formations are produced (Weber, 1933). Beauverie (1938) reports a rather typical series of changes in the chromoplasts of *Ranunculus* petals; there is a granulation and a very marked lipophanerosis or segregation of the plastid colloids. Sections of carrot roots were mounted directly in a one per cent solution of sodium oleate. After thirty-five minutes' treatment there was no change in the appearance of the pigment. A few of the long ribbons showed some fraying of the ends. After forty-five minutes the cytoplasm had become distinctly granular, but the pigment bodies showed no change in structure. They were distinctly embedded in the granular cytoplasm which showed no specialization adjacent to the carotene body. After forty-eight hours' immersion in one per cent sodium oleate, almost complete cellular disorganization had occurred without seeming to affect the pigment bodies in any way. They were still present in considerable quantity. No myelin formations were observed.

Pyridene.—When mounted in a saturated aqueous solution of pyridene, chloroplasts swell and become very granular. Sections of carrot root were treated with pyridene, either by mounting them directly in a saturated aqueous solution or by subjecting them to the fumes and then mounting them in water for observation. No change was ever observed in the structure of the pigment bodies.

Dilute alcohol.—This reagent brings about a swelling followed by a granulation or vesiculation and subsequently a coalescence of the plastids within a cell (Liebaldt, 1913). Dilute alcohols have no

effect upon the appearance of the pigment bodies in the carrot root.

Distilled water.—In distilled water plastids imbibe water, swell and finally burst (Liebaldt, 1913). Distilled water does not change the appearance of the pigment bodies in the carrot.

Alcohol.—While the action of concentrated alcohol has already been treated in some detail, it should be mentioned here that the pigment bodies in the carrot root may be completely dissolved without leaving a trace of a plastid substrate, or a small colorless granule soluble in chloroform may remain as a residue. When chloroplasts are treated with strong alcohol the chlorophyll is dissolved away, but the plastid substrate remains behind as a definite body with all of the structures of the living plastid distinctly visible (Heitz, 1936; Weier, 1936).

While further work may bring to light the plastid nature of the carotene bodies in the carrot root it does seem that the evidence so far available indicates that the pigment is not at all times associated with a typical plastid substratum. In differentiating cells it appears to be diffused throughout the cytoplasm. In many cells containing starch it is closely associated with starch grains. In this state the pigment-starch-cytoplasm complex is probably in the nature of a plastid. In older cells it is present in the form of bodies, crystalline in appearance, which have no plastid attributes.

BEARING OF OBSERVATIONS ON THE EXTRACTION OF PIGMENT FOR CHEMICAL STUDIES.—While the observations do give rather strong support to the contention that the carotene pigment of the carrot root is associated with some other compound of a lipoidal nature they also indicate that great care must be taken in extracting the pigment for such studies. In the first place the association between pigment and substrate is not uniform throughout the root, and the same solvent may not necessarily extract identical compounds from different associations. Secondly, the disorganization of the cytoplasm with the resulting release of cytoplasmic lipoids must be considered. In most instances the pigment appears to dissolve in these lipoids upon the disintegration of the cytoplasm. This association is not that existing in the living cell, yet it might well be the one extracted for study.

It would appear from these observations that the best way to release the free carotene bodies would be by the use of substances such as sodium oleate which bring about a liquefaction of the cytoplasm without an accompanying visible change in the structure of the carotene crystal.

SUMMARY

In differentiating cells of the carrot root the pigment appears either as small crystalline bodies or it is diffused throughout the cytoplasm. These cells contain little or no starch.

In older cells containing starch the pigment is present in the cytoplasm surrounding these grains. It is not always crystalline in appearance. When it

is crystalline, it may sometimes be attached to the starch grains, or it may be free to move away from the grains in the streaming cytoplasm. When these cells are treated with alcohol of 85 per cent or greater concentration large numbers of greenish granules appear in them.

In older cells the starch is withdrawn and large numbers of carotene bodies of a great variety of shapes remain. These bodies are brilliantly doubly refractive between crossed nicols; they reflect blue, green and yellow light with dark field illumination. When these cells are treated with alcohols of a concentration of 85 per cent or less there is no dissolution or change in the appearance of the carotene. When they are treated with 95 per cent or absolute alcohol, the carotene crystals may dissolve completely or a colorless residue which is soluble in chloroform may be left behind.

When sections of carrot are dried, the carotene appears to dissolve in the lipoids released from the cytoplasm. Large numbers of greenish droplets are formed. These may disappear upon standing for twenty-four hours or longer. The carotene does not disappear from sections which have been treated with weak osmic acid and dried or heated to 205°C.

Commercial formol, 2 per cent commercial formol, 2 per cent phenol and approximately .1 mol HCN do not induce great changes in the appearance of the pigment crystals. These substances do, however, render the pigment more resistant to alcohol. Chloroform induces the formation of greenish or yellow droplets.

The pigment-starch-cytoplasmic complex in the carrot root does not react to alcohol, pyridene, sodium oleate, and distilled water as does the typical plastid.

There is good evidence in support of the contention that the carotene is associated with some other substance within the cell. However, it seems certain that upon destruction of the cell the pigment goes into solution in cellular lipoids. Therefore great care should be exercised in the analysis of the so-called carotene-simplex. Unless correct precautions are taken the associated compounds may be those derived from the cytoplasm rather than those normally in association with the pigment.

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SOMATIC CHROMOSOME COMPLEMENTS IN *BOUTELOUA*¹

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CERTAIN SPECIES and biotypes in *Poa* (Brown, 1939; Tinney, 1940), *Paspalum* (Burton, 1940), *Spartina* (Church, 1929a, 1929b and 1940), *Andropogon* (Church, 1929a, 1940), *Agropyron* (Peto, 1930) and other grass genera (Nielsen, 1939) have recently been analyzed cytologically. These investigations have shown that in the above genera, differences between species and between biotypes within species are often correlated with such cytological phenomena as polyploidy, cytomyxis and parthenogenesis. These facts, coupled with the fact that there is much variation in plant form between biotypes of certain species of *Bouteloua*, suggested a study of their chromosome complements to determine whether a similar condition existed in this genus.

An early taxonomic treatment (Griffiths, 1912) of the genus *Bouteloua* described and listed 36 species. The range of the genus is from Ontario and Maine south to the West Indies, the Argentine, and the Galapagos Islands. The center of distribution and the greatest number of species are found in west Texas, southern New Mexico, Arizona and northern Mexico where such species as *Bouteloua rothrockii* Vasey, *B. eriopoda* (Torr.) Torr., *B. aristoides* (H.B.K.) Griseb., *B. barbata* Lag., *B. gracilis* (H.B.K.) Lag. and others often form a dominant part of the vegetation. The species which forms the greatest numerical dominance is undoubtedly the common blue grama (*Bouteloua gracilis*) of the southern, central and northern Great Plains. This species, along with side-oats grama, *B. curtipendula*, has the greatest range of any of the species and these together are typical of the two subdivisions of the genus, the *Chondrosium* and *Atheropogon* sections, respectively.

Since the publication of Griffiths' monograph (1912), Hitchcock (1935) has described the seventeen species occurring in the continental United States. He included five of the species names used by Griffiths within other species. Some of Hitchcock's names were also used by Griffiths but in a more limited sense. Besides these changes, Hitchcock added a brief description of *B. gracilis* var. *stricta* (Vasey) Hitchc. Since the publication of Hitchcock's work, Swallen (1935) has described one new species from California (*Bouteloua annua* Swallen). To these, in

the writer's opinion, should be added one species described by Featherly (1931) from Oklahoma (*Bouteloua pectinata* Featherly). In this report the writer recognizes thirty-three species and one variety based on Griffiths' monograph, and the subsequent work of Hitchcock, Swallen and Featherly.

Of these thirty-three species and one variety, seven species, including six biotypes of blue grama (*B. gracilis*), five of side-oats grama (*B. curtipendula*) and three of hairy grama (*B. hirsuta* Lag.) from numerous seed sources have been studied cytologically. Root tip chromosomes have been observed in one or more biotypes in the following species: *Bouteloua breviseta* Vasey, *B. curtipendula* (Michx.) Torr., *B. eriopoda* (Torr.) Torr., *B. gracilis* (H.B.K.) Lag., *B. hirsuta* Lag., *B. rigidiseta* (Steud.) Hitchc., and *B. rothrockii* Vasey.

The extreme variation in plant type in the widely distributed forms of blue grama and side-oats grama has long been known (Griffiths, 1912). Griffiths acknowledged that the northern plains form of blue grama, which normally produces a rough, bunchy sod, with few culms and abundant basal leaves, is different from the typical southwestern bunch-grass with many culms and culm leaves. However, the fact that both are present in the southwest and that intermediate types occur led Griffiths to the opinion that neither form should be recognized as a distinct variety. Since Hitchcock (1935) did not name or describe these separately, he evidently agreed with Griffiths' interpretation. Hitchcock did concede that *B. gracilis* var. *stricta* (Vasey) Hitchc. is different, and, according to the writer's interpretation of Griffiths (1912), this is typically the tall, robust, strictly bunchy form of the southwest.

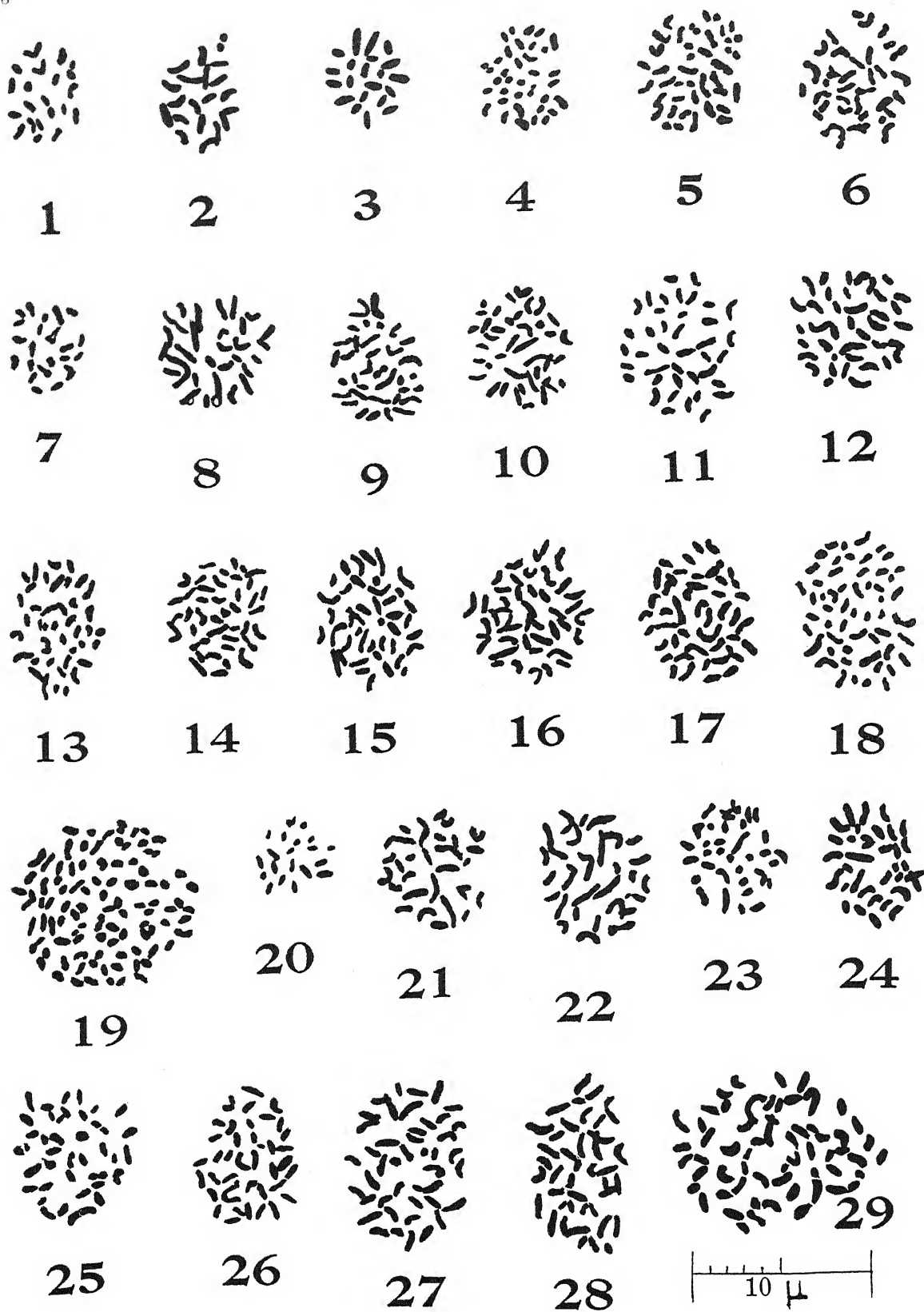
MATERIALS AND METHODS.—The plants used in this investigation were single plant cultures from seed sources ranging from Craigmyle, Alberta, to Iowa, southern Arizona, and New Mexico. One hundred fourteen plants were studied from 85 localities, distributed over every state west of the 100th meridian to the Sierras excepting Idaho, Utah, and Nevada. In *B. breviseta* one plant from one locality was studied; in *B. curtipendula* forty-eight plants representing thirty-eight sources; in *B. eriopoda* five plants from three different areas; in *B. gracilis* fifty-five plants from thirty-eight places of collection; in *B. hirsuta* three plants from three areas; and in *B. rigidiseta* and *B. rothrockii* one plant from one locality each.

All fixations were made with fresh formalin acetic alcohol. The best results were secured by preheating the fixative to 60°C. Root tips killed and fixed on warm bright days gave many more metaphase plates than those taken on cool cloudy days. Little usable material was secured from greenhouse plants growing during the winter months of November through March. The best root tips were obtained during the months of April, May, and early June.

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The author wishes to express his appreciation for the help and assistance given in pursuing the details of this research to Dr. Elda R. Walker, of the Department of Botany of the University of Nebraska, under whose direction it was begun and concluded. Thanks are also due the Nursery Section, Operations Division, Soil Conservation Service, U. S. Department of Agriculture, for the privilege of using certain habitat photographs and for the plant materials used in this study. Thanks are due the Department of Agronomy, the University of Nebraska, for land and greenhouse facilities, and to the Department of Botany, the University of Nebraska, for certain laboratory privileges and materials.



Repeated efforts to use various modifications of the aceto-carmin smear technique failed to give satisfactory results, because either the chromosomes failed to take enough stain or the cytoplasm stained so darkly that differentiation was difficult. Consequently, the paraffin technique was used. Dehydration was done with the various grades of alcohol, followed by chloroform, chloroform-paraffin, and paraffin. Five or six tips were embedded together and then cut at 12 microns. Staining was done with Heidenhain's haematoxylin that was ripened for a period of two months. Two drops of poleol per 100 cc. of stain were added immediately before use. The addition of this latter compound, an agent for lessening surface tension, greatly increased the penetration and contrast of the haematoxylin.

All drawings were made with the aid of a Bausch and Lomb camera lucida and binocular microscope using $\times 25$ oculars and a $\times 98$ objective. The microphotographs were made with a Model K, Bausch and Lomb camera.

In drawing complements with crowded or overlapping chromosomes, it was often possible to bring all chromosomes into one plane, for drawing and photographing, by gentle pressure on the cover slip with the rounded end of the handle of a dissecting needle. In selecting complements to draw and count, five to ten metaphase plates were examined and the best one used. The one selected was always tested by focusing above and below the plate to make certain complete cells were obtained.

Herbarium specimens of all plants used in the investigation have been deposited in the Herbarium of the University of Nebraska.

OBSERVATIONS.—The morphology of chromosomes has proven to be comparatively uniform (fig. 1 to 29) in all species and biotypes. With few exceptions individual chromosomes were spherical or short to medium length rods with a range in size from 0.25 to 2.00 microns. When complete complements were measured, the predominating types were 0.50 to 0.75 micron long and 0.25 to 0.50 micron wide. In a few, definite V-shaped chromosomes with median spindle attachment were observed. In others what appeared

to be strong attraction between certain chromosomes caused complements to appear to be made up of longer and fewer chromosomes than was actually true. When observed at early anaphase, this bunching of chromosomes was not seen and chromosomes were more nearly the usual length and expected number.

BIOTYPES AND CHROMOSOME COMPLEMENTS IN BLUE GRAMA.—As a result of growing blue grama (*B. gracilis*) from 38 different areas side by side in the same nursery, it was possible to classify them into six biotypes. The two most distinct types corresponded closely with the low northern prairie form and the robust southwestern bunch-grass described by Griffiths (1912). Besides these two, four others, somewhat less distinct, have been observed. All are illustrated in figures 49 to 54 and are described and compared below.

The descriptions of six biotypes of blue grama.—(1) *Oklahoma biotype* (fig. 49) closely related to the Lincoln, Nebraska, salt flats form. *Leaves:* Basal, abundant, very long, lax; few or no culm leaves. Average length of basal leaves 22.19 cm. \pm 0.47 cm.² *Culms:* Mostly short and not overtopping leaves. Average number per plant 115.29 \pm 16.05. *Spikes:* Long, narrow, similar to the New Mexico type. Ascending not spreading. Average length of spikes 35.66 \pm 0.87 mm. *Time of flowering:* Late summer to early fall. *Plant vigor:* Very high.

² These averages were obtained from at least ten measurements on each of twenty different individual plants of the biotype concerned, spaced 2 feet \times 2 feet. The errors shown are standard errors (S.E.) and were calculated by the use of the following formula:

$$S.E. = \frac{S.D.}{\sqrt{N}}$$

S.D. = Standard deviation.

N = Total number in population which is 200 or more in this case.

$$S.D. = \sqrt{\frac{\sum X^2}{N} - \left(\frac{\sum X}{N}\right)^2}$$

Significant differences in any one comparison may be detected by determining whether or not they are at least three times the standard errors of the difference which in turn may be calculated by use of the formula:

$$S.E._d = \sqrt{(S.E._1)^2 + (S.E._2)^2}$$

Where $S.E._1$ and $S.E._2$ are the standard errors of the two averages being compared.

Fig. 1-29. Somatic chromosome complements in seven species of *Bouteloua*.—Fig. 1. *B. breviseta* 1386⁵ (2n=21).—Fig. 2. *B. eriopoda* 1607 (2n=21).—Fig. 3-5. Three different complements in *B. hirsuta*.—Fig. 3. *B. hirsuta* 1181 (2n=21).—Fig. 4. *B. hirsuta* 2164 (2n=37).—Fig. 5. *B. hirsuta* 1498 (2n=42).—Fig. 6. *B. rigidisetia* 1977 (2n=35).—Fig. 7. *B. rothrockii* 1385 (2n=22).—Fig. 8-19. Twelve complements in *B. curtipendula*.—Fig. 8. *B. curtipendula* 376 (2n=28).—Fig. 9. *B. curtipendula* 2047 (2n=35).—Fig. 10. *B. curtipendula* 958 (2n=40), total complement length 19.25 microns.—Fig. 11. *B. curtipendula* 2562 (2n=40), total complement length 26.50 microns.—Fig. 12. *B. curtipendula* 373 (2n=40), total complement length 29.25 microns.—Fig. 13. *B. curtipendula* 1179 (2n=42), total complement length 29.75 microns.—Fig. 14. *B. curtipendula* 2158 (2n=42), total complement length 34.75 microns.—Fig. 15 is another *B. curtipendula* 376 (2n=42), total complement length 38.75 microns.—Fig. 16. *B. curtipendula* 2356 (2n=42), total complement length 38.75 microns.—Fig. 17. *B. curtipendula* 2560 (2n=45).—Fig. 18. *B. curtipendula* 1728 (2n=56).—Fig. 19. *B. curtipendula* 2336 (2n=98).—Fig. 20-29. Ten complements of *Bouteloua gracilis*.—Fig. 20. *B. gracilis* 2030 (2n=21).—Fig. 21. *B. gracilis* 2433 (2n=28), total complement length 25.75 microns.—Fig. 22. *B. gracilis* 2038 (2n=28), total complement length 31.75 microns.—Fig. 23. *B. gracilis* 962 (2n=35), total complement length 18.75 microns.—Fig. 24. *B. gracilis* 969 (2n=35), total complement length 29.75 microns.—Fig. 25. *B. gracilis* 2422 (2n=42), total complement length 24.75 microns.—Fig. 26. *B. gracilis* 2325 (2n=42), total complement length 29.00 microns.—Fig. 27. *B. gracilis* 382 (2n=42), total complement length 36.75 microns.—Fig. 28. *B. gracilis* 1180 (2n=42), total complement length 40.00 microns.—Fig. 29. *B. gracilis* 1723 (2n=ca. 61). All magnifications $\times 3,000$.

⁵ These numbers designate source of the seed from which the plant of this drawing was made and are identified in the text.

(2) *Wyoming biotype* (fig. 50) closely related to the Nebraska and North Dakota type. *Leaves*: Basal, medium to short; cauline, medium, both fairly upright. Average length basal leaves 8.08 ± 0.25 cm. *Culms*: Mostly overtopping the basal leaves. Average number of culms per plant 139.00 ± 13.33 . *Spikes*: A little less than aver-

Spikes: Longer than average, horizontally spreading. Average length 35.33 ± 0.32 mm. *Time of flowering*: Mid and late summer, often with two crops. *Plant vigor*: Average.

(4) *Lincoln, Nebraska, salt flat biotype* (fig. 52) closely related to the Oklahoma, and less so to the Nebraska type. *Leaves*: Basal, very long with few cauline leaves. Average

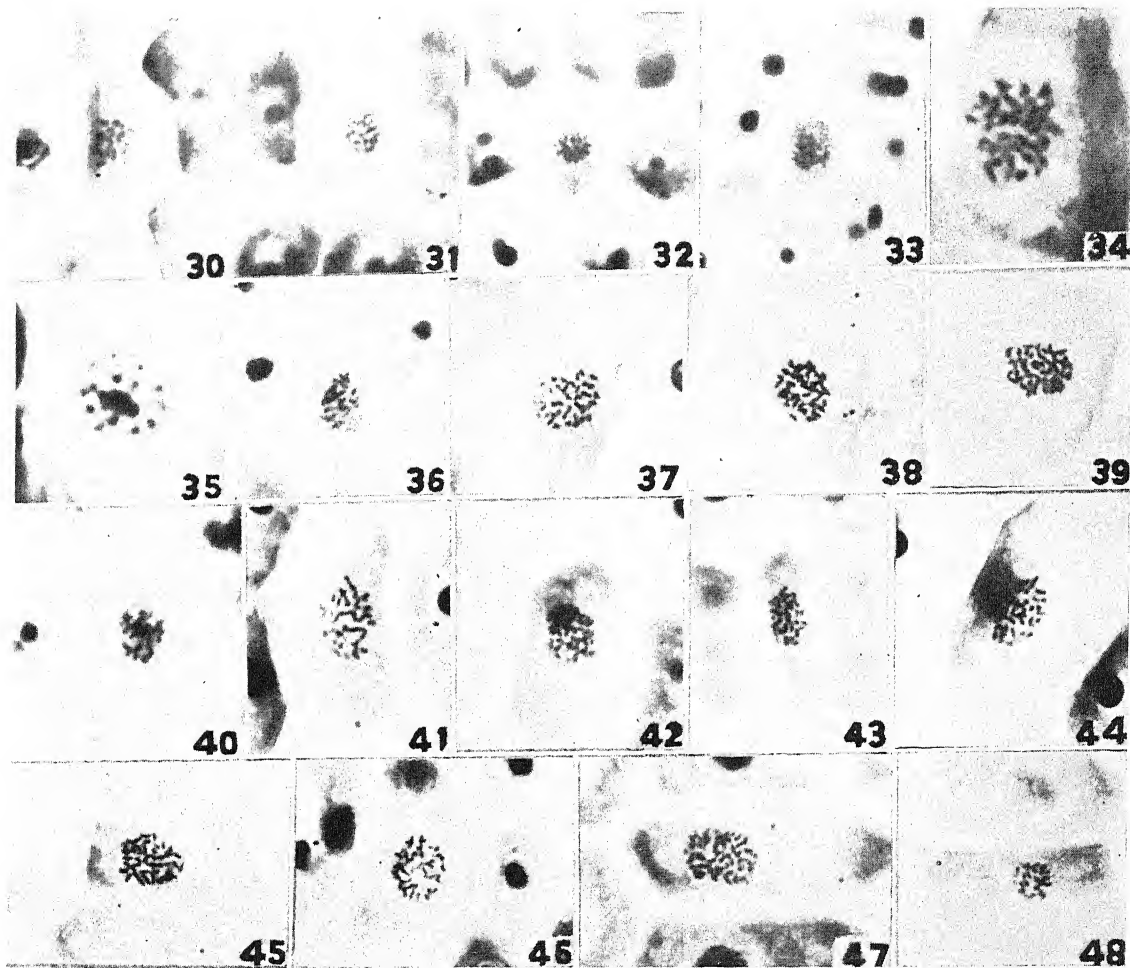


Fig. 30-48. Photomicrographs of various somatic chromosome complements in *Bouteloua*.—Fig. 30. *B. breviseta* 1386 ($2n=21$).—Fig. 31. *B. eriopoda* 1607 ($2n=21$).—Fig. 32. *B. hirsuta* 1181 ($2n=21$).—Fig. 33. *B. hirsuta* 1498 ($2n=42$).—Fig. 34. *B. hirsuta* 2164 ($2n=37$).—Fig. 35. Prochromosomes in a resting nucleus of *B. curtipendula* 376 ($2n=28$).—Fig. 36. *B. curtipendula* 2047 ($2n=35$).—Fig. 37. *B. curtipendula* 2562 ($2n=40$).—Fig. 38. *B. curtipendula* 376 ($2n=28$).—Fig. 39. *B. curtipendula* 2560 ($2n=45$).—Fig. 40. *B. gracilis* 379 ($2n=42$).—Fig. 41. *B. gracilis* 1958 ($2n=28$). Note the strong association of certain chromosomes giving the appearance of a few very long chromosomes.—Fig. 42. *B. gracilis* 2036 ($2n=42$).—Fig. 43. *B. gracilis* 1995 ($2n=42$). Note difference in total complement length between this and fig. 42.—Fig. 44. *B. gracilis* 1971 ($2n=42$).—Fig. 45. *B. gracilis* 2225 ($2n=42$).—Fig. 46. *B. gracilis* 380 ($2n=42$).—Fig. 47. *B. gracilis* 969 ($2n=42$).—Fig. 48. *B. gracilis* 2030 ($2n=21$). All magnifications are $\times 1,200$ except figures 34 and 35 which are $\times 2,400$.

age length, spreading at wide angles to culm. Average spike length 30.87 ± 0.48 mm. *Time of flowering*: Mid and late summer, often with two crops of spikes. *Plant vigor*: Average vigor.

(3) *Nebraska biotype* (fig. 51) most closely related to the Wyoming type. *Leaves*: Much like the Wyoming type, but often somewhat longer. Average length of basal leaves 11.83 ± 0.16 cm. *Culms*: Much like the Wyoming type but not overtopping the leaves quite so distinctly. A little above average number culms per plant 184.24 ± 9.14 .

length of basal leaves 17.67 ± 0.32 cm. *Culms*: A little above average number per plant, almost equaling the Nebraska type and more than the Oklahoma type. Average number per plant 197.30 ± 17.33 . *Spikes*: Long and narrow; often not overtopping the leaves. Often ascending. Average length 36.76 ± 0.53 mm. *Time of flowering*: Mid and late summer, often with two crops. *Plant vigor*: Very high.

(5) *New Mexico biotype* (fig. 53) closely related to *Bouteloua breviseta* Vasey. *Leaves*: Both basal and cau-

line long. Basal leaves not as numerous or as long as in the central Oklahoma type. Average length of basal leaves $16.93 \pm .46$ cm. *Culms*: Many, giving plants a distinctly stemmy habit. Average number of culms per plant 297.61 ± 27.06 . *Spikes*: Long, narrow, similar to those of *B. breviseta*; mostly arched even when immature. Average length 38.35 ± 0.62 mm. *Time of flowering*: Mid to late summer, often two distinct crops. *Plant vigor*: High, especially the rate of increase of basal area.

(6) *North Dakota biotype* (fig. 54) closely related to the Nebraska and Wyoming types. *Leaves*: Cauline, relatively few, spreading at wide angles from the culm. Basal leaves very short. Average length of basal leaves 6.22 ± 0.16 cm. *Culms*: Few per plant (62.55 ± 8.34). Short and distinctly overtopping the basal leaves. *Spikes*: Average length 29.60 ± 0.40 mm., thick and spreading horizontally from the culms. *Time of flowering*: Early summer, with only infrequently a second crop. *Plant vigor*: Poor.

Chromosome complements in blue grama.—After the 38 seed sources were classified into the six biotypes described above and were studied cytologically, an effort was made to correlate the two kinds of information. With one exception, the biotypes have proven not to be directly related to any one chromosome complement.

Triploid complements in blue grama (B. gracilis) 2n=21.—Plants from Antonito, Colorado (2030,³ fig. 53), of the New Mexico biotype were observed with $2n=21$. Since, from complement numbers to be discussed later, the basic number seems to be 7, this seed source is assumed to be a triploid. The chromosomes (fig. 20 and 48) in this plant ranged in length from 0.25 to 0.75 micron with most of the chromosomes being 0.50 micron long.

Tetraploid complements in blue grama (B. gracilis) 2n=28.—The tetraploid number ($2n=28$) was found in plants from nine seed sources. These were Lincoln, Nebraska (1958, fig. 41), Flagstaff, Arizona (2032), Galatea, Colorado (2034), Tucson, Arizona (2038, fig. 22), Hugo, Colorado (2338), Fort Collins, Colorado (2339), Hebron, North Dakota (2424), Kingman, Kansas (2433, fig. 21), and La Vegas, New Mexico (2566).

The biotypes represented in these tetraploids belonged to the Lincoln, Nebraska, salt flat type (1958 and 2433), the New Mexico (2032, 2034, 2038, 2566), the Nebraska (2338, 2339), and the North Dakota type (2424).

Pentaploid complements in blue grama (B. gracilis) 2n=35.—Plants from seven sources were found to be pentaploids ($2n=35$). The plants with this kind of complement were from Cheyenne, Wyoming (383), Ames, Iowa (969, fig. 24), Hartley, Texas (1725), Mandan, North Dakota (2049), Tucumcari, New Mexico (2157), and Limon, Colorado (2332). The biotypes represented were the Wyoming (383), the Nebraska (962, 969 and 2332), the Oklahoma (1725), the North Dakota (2049), and the New Mexico (2157).

Hexaploids and other ploidies in blue grama (B. gracilis).—Plants from twenty sources had the hexa-

ploid count of $2n=42$. These were from North Platte, Nebraska (378), Dickens, Iowa (379), Crystal Lake, Wyoming (380, fig. 46), Crawford, Nebraska (381), Raton, New Mexico (382, fig. 27), Cheyenne, Wyoming (386), O'Neill, Nebraska (969, fig. 47), Stillwater, Oklahoma (1180), Teton County, Montana (1524), Whitehall, Montana (1969), Mandan, North Dakota (1971, fig. 44), Craignyle, Alberta (1982), Mullen, Nebraska (1995, fig. 43), Las Cruces, New Mexico (2036, fig. 42), Hartley, Texas (2155), Mexican Springs, New Mexico (2156), New Amsterdam, Montana (2225, fig. 45), Center, North Dakota (2422), Killdeer, North Dakota (2423), and Julesburg, Colorado (2563). Five different biotypes were represented as follows: the Nebraska (378, 379, 1995), the Wyoming (380, 381, 386, 2563), the New Mexico (382, 2036, 2155, and 2156), the North Dakota (1524, 1969, 1971, 1982, 2225, 2422 and 2423) and the Oklahoma (1180).

Plants from but two sources have been found with chromosome numbers greater than $2n=42$. These were found in a plant from Enid, Oklahoma (1723, fig. 29) with $2n=61$, and a plant from Stillwater, Oklahoma (1724) with $2n=77$. Both plants were of the typical Oklahoma biotype.

The morphology of the chromosomes in blue grama.—Since the chromosomes in this species were so small, no definite morphological characters identifying them, such as constrictions and satellites, could be detected. Distinct differences in lengths of chromosomes in plants from the different seed sources did occur. In an attempt to classify these differences, at least one or more complete complements were drawn and measured for each seed source. The results of these measurements are shown in table 1.

There is no significant difference in the average complement length between the tetraploids and pentaploids, but there is a distinct difference between the mean length of the hexaploids and either the tetraploids or the pentaploids as shown in table 1. In all three ploidies, chromosomes measuring 0.75 micron were most frequent. The longest chromosomes (1.75 to 2.00 microns) were found in the tetraploid (fig. 22). The differences in total chromosome length within a given ploid are illustrated in figures 21 and 22. In figure 21 (2433 Kingman, Kansas) the total length of the 28 chromosomes is 25.75 microns; in figure 22 (2038 Tucson, Arizona) the total length of the twenty-eight chromosomes is 31.75 microns. Similar differences in the pentaploids are shown in figure 23 (962 Ames, Iowa) with a total length of 29.75 microns. Figures 25, 26, 27 and 28 show similar increasing total complement lengths in hexaploids.

BIOTYPES AND CHROMOSOME COMPLEMENTS IN SIDE-OATS GRAMA.—The chromosome complements of plants from 32 seed sources of side-oats grama (*B. curtipendula*) were studied. When grown in the same nursery it was possible to classify the plants from all sources into five distinct biotypes. These are designated in this report as the Temple, Texas (fig.

³ These numbers are the accession numbers of the Nursery Section, Operation Division, Soil Conservation Service, U. S. Department of Agriculture, Lincoln, Nebraska.

TABLE 1. *Chromosome lengths in blue grama (B. gracilis).*

Soil Conservation Service accession number and source	0.25 mic.	0.50 mic.	0.75 mic.	1.00 mic.	1.25 mic.	1.50 mic.	1.75 mic.	2.00 mic.	Total length in microns
<i>Complements with 28 chromosomes</i>									
1958, Lincoln, Nebraska, salt flats...	5	2	12	4	3	2	22.00
2433, Kingman, Kansas	10	7	1	4	4	2	..	25.75
2424, Hebron, North Dakota	7	9	4	4	2	1	1	26.00
2338, Hugo, Colorado	4	10	8	4	2	26.50
2339, Fort Collins, Colorado	8	6	3	2	5	3	1	28.75
2566, Las Vegas, New Mexico	1	8	11	2	5	1	..	29.25
2038, Tucson, Arizona	2	11	1	4	5	4	1	31.75
Average	0.71	4.85	9.00	4.57	3.28	3.28	1.57	0.71	27.14
<i>Complements with 35 chromosomes</i>									
962, Ames, Iowa	10	15	7	1	2	18.75
383, Cheyenne, Wyoming	7	20	1	4	3	29.00
2332, Limon, Colorado	10	12	5	6	2	29.50
2049, Mandan, North Dakota	1	10	12	4	3	5	29.50
969, O'Neill, Nebraska	2	8	14	5	5	1	29.75
2157, Tucumcari, New Mexico	12	9	3	7	4	30.50
Average	2.16	10.33	12.33	3.16	4.50	2.50	27.83
<i>Complements with 42 chromosomes</i>									
2422, Center, North Dakota	6	18	15	3	24.75
1995, Mullen, Nebraska	6	19	12	3	2	25.50
381, Crawford, Nebraska	3	17	16	6	27.25
2225, New Amsterdam, Montana	4	8	26	2	2	29.00
378, North Platte, Nebraska	1	10	26	3	2	30.25
2034, Galatea, Colorado	1	15	16	6	2	2	30.25
2036, Las Cruces, New Mexico	6	7	17	10	1	1	30.50
1971, Mandan, North Dakota	2	13	18	3	2	4	32.00
1982, Craigmyle, Alberta	12	19	2	9	33.50
1969, Whitehall, Montana	1	7	25	4	1	4	33.75
2156, Mexican Springs, New Mexico	13	15	8	5	1	33.50
2423, Killdeer, North Dakota	1	9	18	4	7	3	35.50
382, Raton, New Mexico	4	24	6	5	3	36.75
2155, Hartley, Texas	9	18	5	4	6	37.00
380, Crystal Lake, Wyoming	2	10	11	4	9	6	38.00
1180, Stillwater, Oklahoma	10	10	7	8	7	40.00
Average	2.06	11.31	17.87	4.75	3.68	2.31	32.34

60), the Nebraska (fig. 63), the Oklahoma (fig. 64), the southern Arizona (fig. 65), and the North Platte, Nebraska, biotypes (fig. 66). These appeared to have more intermediates than those described for blue grama (*B. gracilis*), but each was readily recognized when growing side by side under cultivation. The differences and similarities are described and compared below.

The descriptions of five biotypes of side-oats grama.—

(1) *Temple, Texas, biotype* (fig. 60). *Leaves*: Mostly basal and very long, cauline, long and coarse. Average length of basal leaves 26.28 ± 0.75 cm.⁴ *Culms*: Few in number and short, barely overtopping leaves, internodes short. *Inflorescence*: Often not exerted, those emerging well filled with spikes. *Plant vigor*: Especially high first two seasons.

(2) *Nebraska biotype* (fig. 63) closely related to the Oklahoma type. *Leaves*: Of average length and abun-

⁴ These are means and standard errors for use in determining significant differences by use of the same formula discussed before for blue grama.

dance, about equally distributed between cauline and basal leaves. Average leaf length 17.28 ± 0.38 cm. *Culms*: Average number per plant, these distinctly overtopping the leaves. Average height of culms 57.17 ± 0.79 cm. *Inflorescence*: Average length 23.20 ± 0.48 cm. Well filled with spikes. *Plant vigor*: Average.

(3) *Oklahoma biotype* (fig. 64) closely related to the Nebraska type. *Leaves*: Of average length, comparable to the Nebraska type but leaves coarser and more abundant. Average length 17.27 ± 0.41 cm. *Culms*: Many per plant, compared to the Nebraska type, these usually longer. Average height 39.71 ± 1.13 cm. *Inflorescence*: Average length 25.22 ± 0.61 cm.; well filled with long spikes. *Plant vigor*: Above average.

(4) *Arizona biotype* (fig. 65). *Leaves*: Average number of long culm leaves, basal ones sparse, weak and lax. Average length 15.65 ± 0.26 cm. *Culms*: Many, very long, weak, often geniculate at the base, spreading. Average height 73.65 ± 1.13 cm. Long internodes. *Inflorescence*: Long with long spikes loosely arranged. Average length 24.08 ± 0.43 cm. *Plant vigor*: High first year but not winter hardy in Nebraska.

(5) *North Platte, Nebraska biotype* (fig. 66). *Leaves*: Basal few and short, very few short culm leaves. Average leaf length 9.44 ± 0.49 cm. *Culms*: Shorter and fewer than average, distinctly overtopping the leaves. Average height 42.52 ± 0.96 cm. *Inflorescence*: Short, average length 13.21 ± 0.54 cm. *Plant vigor*: Very weak.

The chromosome complements in side-oats grama.—After plants from the thirty-two seed sources of side-oats grama had been studied cytologically, the results were related to the five biotypes. As in blue grama the biotypes were not found to have distinctly characteristic chromosome complements.

Hexaploid and near hexaploid complements in side-oats grama.—Plants from four sources had aneuploid complements of $2n=40$. These were from O'Neill, Nebraska (373, fig. 12), McGregor, Iowa (958, fig. 10), El Reno, Oklahoma (1497), and Seiling, Oklahoma (2562, fig. 11 and 37). Two biotypes were represented, the Nebraska (373 and 958) and the Oklahoma (1497 and 2562).

Hexaploids ($2n=42$) were found in plants from eighteen sources. These were Algona, Iowa (374), O'Neill, Nebraska (375), Kuippa, Texas (1179, fig. 13), Crockett County, Texas (1730), Wanoka, Oklahoma (1732), Aztec, New Mexico (2029), Beulah, Colorado (2035), Hays, Kansas (2158, fig. 14), Oklahoma (2163), Harmon, North Dakota (2335), Colorado Springs, Colorado (2343), Clinton, Oklahoma (2356, fig. 16), El Reno, Oklahoma (2358), Stillwater, Oklahoma (2359), Columbus, Nebraska (2409), Lindsborg, Kansas (2412), Altoona, Kansas (2558), and Temple, Texas (2561). These

plants all belonged to but two biotypes, the Nebraska (374, 375, 376, 971, 1732, 2035, 2335, 2409, 2412 and 2558) and the Oklahoma (1730, 2158, 2163, 2343, 2356, 2358 and 2359).

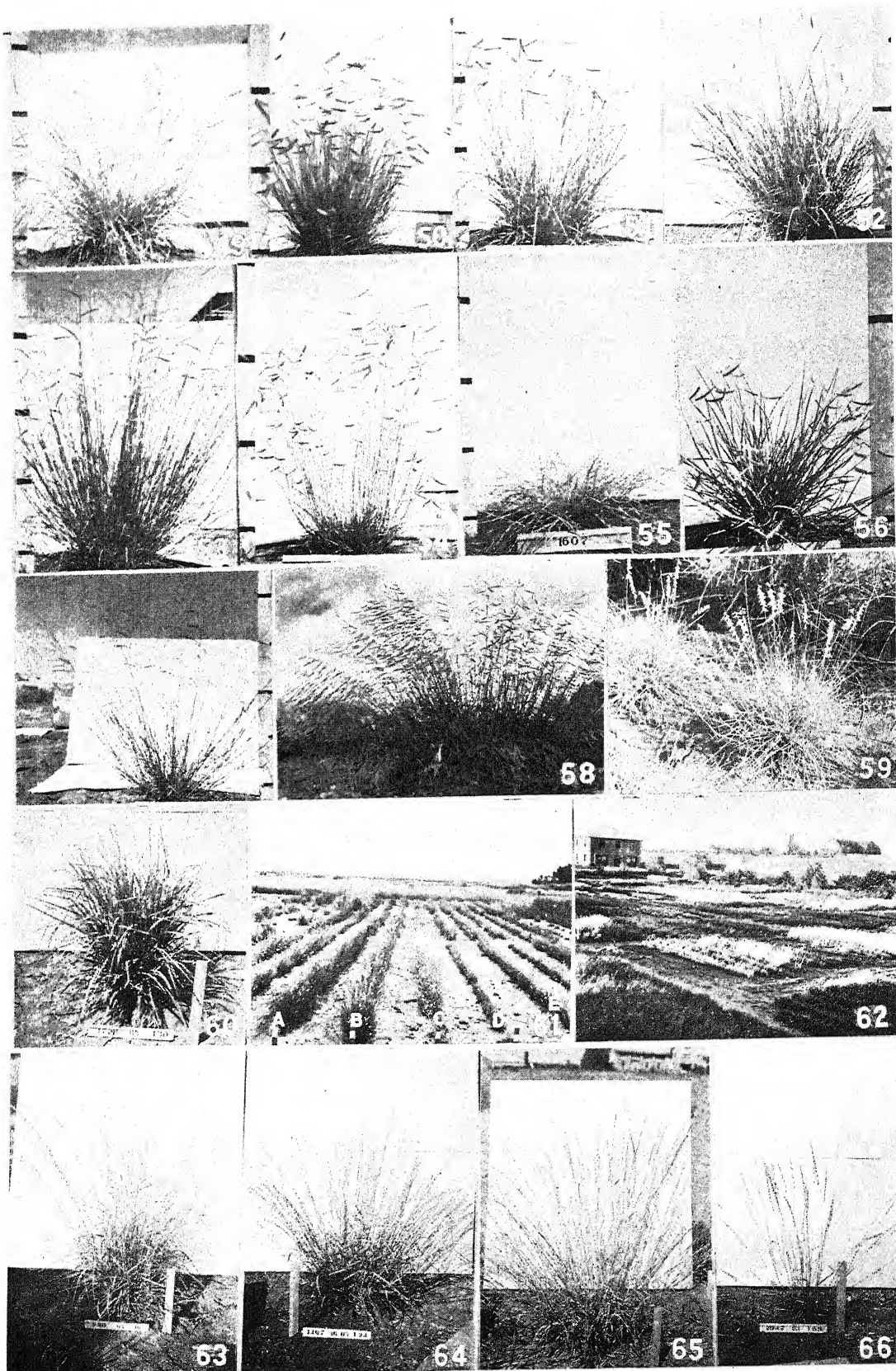
Polyploid types higher than the hexaploid in side-oats grama.—One aneuploid type was found with 45 chromosomes (fig. 17). This plant came from Chickasha, Oklahoma (2560), and belonged to the Oklahoma biotype.

One plant from Temple, Texas (1728), with $2n=56$ (fig. 18) was found. This plant belonged to a distinctive type called here the Temple, Texas, biotype (fig. 60). Two plants, one from Whitset, Texas (1729), and one from Flagstaff, Arizona (2028), were found with $2n=70$. The plant from Whitset belonged to the same biotype as the Temple, Texas, plant (fig. 60), and the Flagstaff plant had the characteristics of the southern Arizona biotype (fig. 65). One plant with $2n=98$ (fig. 19) from Galatea, Colorado (2336), was intermediate between the Nebraska and the Arizona biotypes.

The morphology of the chromosomes in side-oats grama.—Differences in total length of chromosomes per complement were found as commonly in side-oats grama as in blue grama. Table 2 shows the tabulated measurements of the complements in four aneuploid plants and sixteen hexaploids. From this table it is evident that the average total length of chromosomes in the aneuploids ($2n=40$) and the hexaploids differs by 5.18 microns. In both types of complement, most of the chromosomes were either 0.50 mi-

TABLE 2. *Lengths of chromosomes in side-oats grama (Bouteloua curtipendula).*

Soil Conservation Service accession number and source	0.25 mic.	0.50 mic.	0.75 mic.	1.00 mic.	1.25 mic.	1.50 mic.	1.75 mic.	2.00 mic.	Total length in microns
<i>Complements with 40 chromosomes</i>									
958, McGregor, Iowa	5	15	6	8	4	2	19.25
1497, El Reno, Oklahoma	3	21	11	2	1	2	25.75
2562, Seiling, Oklahoma	2	20	13	1	3	1	26.50
373, O'Neill, Nebraska	3	12	13	9	3	29.25
Average	3.25	17.00	10.75	5.00	2.75	1.25	25.18
<i>Complements with 42 chromosomes</i>									
2561, Temple, Texas	7	19	14	2	23.75
1732, Wanoka, Oklahoma	3	25	12	1	1	24.50
2163, Oklahoma	8	14	17	2	1	25.00
375, O'Neill, Nebraska	9	13	13	6	1	25.75
1179, Kuippa, Texas	2	25	10	3	2	26.00
2012, Nowata, Oklahoma	3	16	16	7	27.75
2158, Hays, Kansas	1	17	16	4	4	29.75
2035, Beulah, Colorado	3	12	17	5	5	30.75
971, O'Neill, Nebraska	4	13	11	10	3	1	31.00
2358, El Reno, Oklahoma	1	14	17	5	2	3	32.00
2343, Colorado Springs, Colorado	3	8	21	6	4	32.50
1730, Crockett County, Texas	10	21	6	5	33.00
374, Algona, Iowa	10	21	3	8	33.75
376, Norton, Kansas	3	7	18	5	6	3	34.75
2029, Aztec, New Mexico	1	6	19	9	3	2	2	..	36.75
2356, Clinton, Oklahoma	5	19	6	8	4	38.75
Average	3.62	14.04	15.42	4.93	3.00	0.81	0.12	..	30.36



cron or 0.75 micron long. No chromosomes longer than 1.75 microns were found.

The differences in total length of chromosomes within the $2n=40$ type are illustrated in figure 10 (McGregor, Iowa, 958; total length 19.25 microns), figure 11 (Seiling, Oklahoma, 2562; total length 26.50 microns) and figure 12 (O'Neill, Nebraska, 373; total length 29.25 microns). In the hexaploids four plants are illustrated (fig. 13, 14, 15 and 16). Each succeeding complement differs from the one preceding by at least 4 microns.

CHROMOSOME COMPLEMENTS IN FIVE ADDITIONAL SPECIES.—In *B. breviseta*, it was found that the chromosome number was $2n=21$ (fig. 1 and 30). The plant from which this count was made (fig. 57) was typical of the species with its many stems, few basal leaves, long culm leaves, and long spikes.

Plants of *B. eriopoda* from three different seed sources were investigated and all had $2n=21$ chromosomes. The seed sources were Tucson, Arizona (1607), Las Cruces, New Mexico (2036), and Belen, New Mexico (2154). The plant from Tucson, Arizona (1607) had chromosomes ranging in length from 0.25 to 1.50 microns with an average length of 1.00 micron (fig. 2 and 31), which is considerably longer on an average than those counted in *B. breviseta*. These plants also had two small spherical chromosomes in each complement (fig. 2). These appeared to be characteristic.

The plants from the three seed sources of *B. hirsuta* studied had $2n=21$ in the Hebronville, Texas, $2n=37$ in the Hays, Kansas (2164), and $2n=42$ in the Stillwater, Oklahoma (1498) biotypes. The Hebronville complement (fig. 3 and 32) had chromosomes ranging in length from 0.50 to 1.50 microns, with the 0.75 micron class most frequent.

Differences in the combined length of all the chromosomes in each complement were shown by the fact that the total length in the Hebronville, Texas, plant was 18.00 microns; in the Hays, Kansas, plant 22.25 microns; and in the Stillwater, Oklahoma, plant 31.50 microns.

The one plant of *B. rigidiseta* studied was from Denton, Texas (1977). It had $2n=35$ chromosomes (fig. 6 and 59). These ranged in size from 0.25 to 2.00 microns, most of which were 1.00 micron long. The total chromosome length of the 35 chromosomes was 31.75 microns.

In the one plant of *B. rothrockii* from Tucson, Arizona (1385), 22 chromosomes were found (fig. 7 and 58). These had much the same morphology as those found in *B. breviseta* (fig. 1).

PROCHROMOSOMES IN BLUE GRAMA AND SIDE-OATS GRAMA.—Although exhaustive investigations of prochromosomes in these species have not been made, a few definite observations can be reported here. Almost all resting cells in the root tips of both species are characterized by distinct prochromosomes. These bodies vary in shape from small spherical bits of chromatin about 0.20 micron in diameter to dumbbell-shaped bodies and short rods 1.25 microns long. Their shape seems to depend on how recently the nuclei of which they are a part have undergone mitosis. In all cases observed the prochromosomes were about equally distributed about the periphery of the nucleus. They were easily counted and drawn, but an accurate count was difficult because the nucleolus often hid one to several.

In one plant of blue grama from Teton County, Montana (1524), with a metaphase root tip count of $2n=42$, 34 spherical to rod-like prochromosomes varying in length from 0.25 to 1.25 microns were drawn and measured. In another plant from Dickens, Iowa (379), with a metaphase root tip count of $2n=42$, 35 spherical to dumbbell-shaped prochromosomes were observed. In the first, the nucleolus measured 3.00 microns in diameter and in the second 3.50 microns, either of which doubtless shielded five or six prochromosomes.

In one plant of side-oats grama from Norton, Kansas (376), with a metaphase root tip count of $2n=28$ (fig. 8), 28 nearly spherical prochromosomes (fig. 35) were observed. In another plant from Wanoka, Oklahoma (1732), with a metaphase root tip count of $2n=42$, 38 prochromosomes were present. In both these cases the prochromosomes were spherical bodies 0.20 to 0.30 micron in diameter.

DISCUSSION.—The studies reported in this paper on the biotypes of blue grama agree with those of Griffiths (1912) concerning the distinctness of the northern low sod type form and the tall vigorous bunchgrass typical of the lower elevations in the southwest. The observations made here do not agree with those of Griffiths regarding the similarity of the two forms under cultivation. In this study, individual plants of the two have maintained their individuality

Fig. 49-66.⁶ Six biotypes commonly observed in *Bouteloua gracilis* (H.B.K.) Lag.—Fig. 49. The Oklahoma biotype.—Fig. 50. The Wyoming biotype.—Fig. 51. The Nebraska biotype.—Fig. 52. The Lincoln, Nebraska, salt flats biotype.—Fig. 53. The New Mexico biotype.—Fig. 54. The North Dakota biotype.—Fig. 55. *Bouteloua eriopoda* (Torr.) Torr.—Fig. 56. *B. hirsuta* Lag.—Fig. 57. *B. breviseta* Vasey.—Fig. 58. *B. rothrockii* Vasey.—Fig. 59. *B. rigidiseta* (Steud.) Hitchc.—Fig. 60. The Temple, Texas, biotype of *Bouteloua curtipendula* (Michx.) Torr.—Fig. 61. This picture illustrates the influence of seed source on the biotype of *Bouteloua gracilis*. The sources of these plants are (A) McPherson, Kansas (2660, Lincoln salt flat biotype); (B) Tucumcari, New Mexico (2157), and (C) Raton, New Mexico (2325), both New Mexico biotype; (D) Center, North Dakota (2422, North Dakota biotype); and (E) O'Neill, Nebraska (2402, Nebraska biotype).—Fig. 62. Grass Garden at Lincoln, Nebraska, supported by Soil Conservation Service, U. S. Department of Agriculture and Department of Agronomy, University of Nebraska, where much of this work was done.—Fig. 63. The Nebraska biotype of *B. curtipendula*.—Fig. 64. The Oklahoma biotype of *B. curtipendula*.—Fig. 65. The Arizona biotype of *B. curtipendula*.—Fig. 66. The North Platte, Nebraska, biotype of *B. curtipendula*.

⁶ Photographs furnished through the courtesy of the Soil Conservation Service, U. S. Department of Agriculture. The marks on the rule in figure 49-57 are 6 inches apart.

under cultivation for five years. The chromosome complements in the two forms tend to be different, but there are many exceptions. As a rule the northern form tends to be hexaploid, while the southwestern one is often tetraploid or pentaploid. One triploid has been found also.

In addition to the northern low sod form and the southwestern bunch grass already discussed, four other biotypes have been described and studied cytologically in this report. Of these, the Wyoming and Nebraska types are most closely related to that of the northern prairie. The other two (the Oklahoma and Lincoln, Nebraska, biotypes) appear to be entirely distinct from either the northern or southwestern plants but are closely related to each other. Both are strong bunch grasses but with unusually long basal leaves. Cytologically, these two types are unstable and tend to have high polyploid chromosome numbers.

The fact that plants grown from different seed sources produce distinctive plants as reported by Savage (1939) and Hoover (1939) agrees with observations made here. This fact indicates a high degree of adaptation to particular habitats and is a factor that should be carefully considered in the domestication of such species as side-oats grama and blue grama.

Since plants grown from seed from different localities are distinctive and are able to maintain their peculiar characters for as long as five years when grown in one environment, their chromosomes undoubtedly have structural differences. Just what these differences are can only be analyzed after a critical examination of meiotic behavior. This awaits further study.

Griffiths (1912) noticed but did not describe the variation in side-oats grama between plants from different areas. This has been substantiated by the writer. Besides, all the plants from a wide range have been classified and grouped into five typical biotypes. These have been studied cytologically. Since this has shown that the five biotypes do not have individually characteristic chromosome complements, it is assumed that differences between biotypes may be due to factors within individual chromosomes. However, a critical analysis of meiosis in the pollen mother cells will be necessary to discern adequately these differences.

The predominance of the low chromosome numbers ($2n=21$) in *B. breviseta*, *B. eriopoda*, *B. hirsuta* and *B. rothrockii* ($2n=22?$) coupled with the fact that these are often dominant species in the southwestern states and northern Mexico is further evidence that this area is near the center of distribution for the genus. The triploid New Mexico biotype of blue grama (*B. gracilis* 2030) from Antonito, Colorado, is closely related to the other southwestern species through *B. breviseta* inasmuch as both have similar growth habits and the same number of chromosomes ($2n=21$). Since only a relatively few plants representative of these species have been

studied, it is entirely possible that variation in chromosome numbers within these species would be found by intensive search.

The hexaploids in both blue grama and side-oats grama are the most common ploidies, the most aggressive and the most widely distributed.

It is also significant that in both species, the few higher ploidies found came from either central Oklahoma, Texas or southern Arizona. This indicates that the production of new types through various chromosomal aberrations is probably still a dynamic process in this region.

The hexaploid count of $2n=42$ found by the writer in the Nebraska biotypes of both blue grama and side-oats grama substantiates the earlier reports of Humphrey (1937) and Neilsen and Humphrey (1937) made on plants grown from seed from the Great Plains.

The fact that most chromosome complements studied by the writer as well as by others have been multiples of 7, would indicate that this is the basic number in the genus. Further study of plants with larger chromosomes, in which the constrictions can be seen, is needed definitely to substantiate this. Since tetraploids in both blue grama and side-oats grama ($2n=28$) have been found, it is likely that the diploid type of $2n=14$ will be found by further search especially in the southwestern biotypes.

The common occurrence of prochromosomes in resting cells of both meristematic and differentiated tissues in blue grama and side-oats grama should prove of considerable value to the plant breeder if it can be conclusively shown that their number is the same as the number of chromosomes. The observations reported here would indicate that such may be the case and agree with earlier observations by other workers on other genera with small chromosomes (Eichhorn, 1931; Doutreligne, 1933; Raghaven, 1938; Smith, 1934; and Zeeuw, 1935).

SUMMARY

The somatic chromosome complements in the genus *Bouteloua* have been investigated in eighteen biotypes belonging to seven species. One hundred fourteen plants from 85 seed sources were included in the study.

Chromosome numbers varied from $2n=21$ to $2n=98$.

The chromosome numbers found in the seven species are: *Bouteloua breviseta* Vasey, $2n=21$; *Bouteloua curtipendula* (Michx.) Torr. $2n=28, 35, 40, 42, 45, 56, 70$ and 98 ; *Bouteloua eriopoda* (Torr.) Torr. $2n=21$; *Bouteloua gracilis* (H.B.K.) Lag. $2n=28, 35, 42, 61$ and 77 ; *Bouteloua hirsuta* Lag. $2n=21, 37$ and 42 ; *Bouteloua rigidiseta* (Steud.) Hitchc. $2n=35$ and *Bouteloua rothrockii* Vasey $2n=22$.

Six biotypes of blue grama (*B. gracilis*) have been described and their chromosome complements studied. No definite relation between complements and

biotypes was found. One biotype often had two or more different chromosome complements.

All the chromosomes in one or more complements of seven tetraploid, six pentaploid and sixteen hexaploid plants of blue grama (*B. gracilis*) were measured. Chromosome lengths varied from 0.25 to 2.00 microns, most of which were 0.50 to 0.75 micron.

The average total chromosome length per complement in blue grama varied from 27.14 microns in the tetraploids and 27.83 microns in the pentaploids to 32.34 microns in the hexaploids. In the same ploid, total lengths differed by as much as 9.75 microns in the tetraploids, 11.75 microns in the pentaploid and 15.25 microns in the hexaploid.

Five biotypes of side-oats grama (*B. curtipendula*) have been described and related to their chromosome complements. No direct correlation was found.

All the chromosomes in one or more complements of four aneuploid ($2n=40$), and sixteen hexaploid ($2n=42$) plants of side-oats grama were measured. Chromosome lengths varied from 0.25 to 1.75 microns most of which were 0.50 to 0.75 micron. The average total chromosome length per complement in the aneuploid ($2n=40$) was 25.18 microns; in the hexaploid ($2n=42$) 30.36 microns. In the same ploid total lengths varied by as much as 10.00 microns in the aneuploid ($2n=40$) and 15.00 microns in the hexaploid.

Prochromosomes in resting cells in both meristematic and differentiated tissue were found to be common. This was especially true in both blue grama (*B. gracilis*) and side-oats grama (*B. curtipendula*). The limited observations made indicated that the number of prochromosomes equals the number of chromosomes.

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STUDIES OF THE GROWTH OF POLLEN WITH RESPECT TO TEMPERATURE, AUXINS, COLCHICINE AND VITAMIN B₁¹

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ALTHOUGH NUMEROUS reports have been made in recent years concerning the influence of various chemical stimulants on plants, relatively few have dealt with the germination and growth of pollen. Apparently no one has mentioned the inter-relation of temperature and chemical stimulation in connection with pollen physiology. In the writer's experience with the culture of pollen certain facts have been brought out concerning growth stimulation by means of chemicals and temperature, as well as the inter-relationship of these two factors. Important factors in the technique of culturing pollen also have come to light. It is the purpose of the present paper to report some of these findings.

MATERIALS AND METHODS.—The pollen of *Lathyrus odoratus* (sweet pea), *Tradescantia occidentalis* (spiderwort), *Antirrhinum majus* (snapdragon) and *Bryophyllum Daegremontianum* were used as test material in preliminary work. Inasmuch as the responses of all four species were essentially similar in most respects, extensive studies were made only with the latter two (*A. majus* and *B. Daegremontianum*), for which data, figures and discussion will be presented here.

Although many of the physiological studies of pollen in the past have been made by the hanging-drop technique (Brink, 1925; Vom Berg, 1929; Cooper, 1939; Smith, 1939), later experiments have shown that the growth of pollen is seriously handicapped by this method. It was found, for instance, that with growth periods of four hours in hanging drops the tube length of the pollen of *Tradescantia occidentalis* was only about one-half as great as that on sugar-agar. Consequently, the sugar-agar technique (Eigsti, 1940a) was used throughout the following experiments. Since a concentration of 0.75 per cent agar was found adequate to support the pollen grains and tubes on the slides, this concentration was used with the appropriate amount of sucrose. For snapdragon a 10 per cent sucrose solution proved satisfactory and with *Bryophyllum* a 3 per cent solution was used.

In choosing pollen, fresh flowers in the late bud stage were brought into the laboratory in the evening. Such flowers were usually at the proper stage for use the next day. Thus it was possible to avoid obtaining old pollen and to prevent the loss of pollen caused by handling the flowers after dehiscence had taken place. The snapdragon transpired so rapidly that it was necessary to keep it under a par-

tially closed bell jar to prevent wilting and scattering of pollen in the dry atmosphere of the room.

Previous workers have encountered fluctuation in the growth responses of pollen. Brink (1924a) mentions the "unexplained vagaries" and "variabilities in the growth reactions of pollen." In the present work, great variation was noted unless special attention was given to the selection of pollen. A large number of slides were inoculated by using the pollen from a single anther for each slide. Two types of variation were found: (1) pollen of different flowers might differ in quality or magnitude of response, and (2) pollen from different anthers of the same flower might give different responses. In snapdragon, little or no variation was observed in the responses of the pollen from the anthers of a single flower. In *Bryophyllum*, however, pollen from one of the eight anthers was frequently partially sterile and gave poor germination. Consequently, pollen from all the anthers of one flower of this species was combined and inoculation made from the mixture. Thus, in either case a single flower furnished the pollen for each test series (*i.e.*, the three auxins, the colchicine, the vitamin B₁ and the control slides). Such a procedure should make different responses within any one series attributable solely to the effect of the test substances in the media. In attempting to reproduce such a series of tests, in order to obtain similar results with other flowers, the variability between individual flowers was encountered. Pollen from flowers of a comparable age (as judged from the time of anther dehiscence) is much more likely to give uniform responses than if their age is disregarded. Apparently there is a time in the development of pollen when all metabolic functions harmonize in such a way that germination and growth of tubes will result if the grain is in a suitable environment. This stage in the physiological development may precede, coincide with, or follow dehiscence. In some species (*e.g.*, periwinkle [*Vinca rosea*]) the pollen often germinates within the anthers. Snapdragon pollen gives best germination shortly after dehiscence and becomes what we may term "old" pollen within three to four hours. *Bryophyllum* possesses a waxy pollen which germinates most satisfactorily several hours after dehiscence and retains the ability to germinate for forty-eight hours or more.

The significant thing about the age factor is that best germination and growth of tubes took place only within these age limits. In snapdragon, young pollen (pollen from flowers which were expanded but with anthers undehiscent) germinated poorly, yielding a very low percentage of germination. The resulting tubes grew slowly and unevenly and never attained the length ordinarily reached by ripe pollen. The tubes from such pollen were extremely

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crooked and exhibited a strong tendency to burst after about two hours' growth. Old pollen (pollen from anthers which had dehisced at least four to six hours previous to inoculation in the case of snapdragon) invariably gave difficulty, at least in quantitative work. Such pollen was characterized by a low percentage of germination and short, extremely crooked tubes.

The majority of flowers chosen by this procedure furnished pollen that gave reasonably uniform responses. It became possible to tell by examining the control slide whether the pollen was at the proper stage of development to give optimum growth. Thus, by using one flower for a complete series and by using

out favorable stimulation in more dilute solutions. As an arbitrary measure a 1:25,000 dilution was chosen for further tests.

Colchicine was used in a 1:10,000 (0.01 per cent) concentration with the pollen of snapdragon, chiefly because this dilution is frequently used in cytological studies. In certain tests with *Bryophyllum*, however, other concentrations were used. In the 25°C. and 35°C. test series with this pollen, three concentrations were used (1:1,000, 1:10,000 and 1:20,000).

The time allowed for growth was chosen after preliminary work with the pollen, and its selection was based on the length attained by the tubes. The experiments were stopped before the tubes grew too

TABLE 1. *Effects of various concentrations of 3-indole acetic acid and vitamin B₁ on the germination and tube length of snapdragon pollen.*

	Concentration of test substance	No. of grains counted	Percentage of germination	Mean tube length in microns
Control	370	58.7	355
3-indole acetic	1: 12,500	330	15.0	97
3-indole acetic	1: 25,000	384	49.5	291
3-indole acetic	1: 50,000	363	59.0	365
3-indole acetic	1:100,000	391	66.1	448
3-indole acetic	1:200,000	377	64.3	423
3-indole acetic	1:400,000	389	63.2	411
Vitamin B ₁	1: 10,000	396	27.8	161
Vitamin B ₁	1: 25,000	335	59.2	339
Vitamin B ₁	1: 50,000	408	58.1	357
Vitamin B ₁	1:100,000	379	58.9	347

many series it was possible to discard the occasional series from flowers which were not of correct physiological age. With these precautions one could be relatively certain that the measured series were showing reactions to the conditions under which they were grown (i.e., presence or absence of the test substances or growth at particular temperatures) and that these reactions did not result from varying physiological conditions of the pollen. Where such precautions were neglected, there was neither harmony nor regularity in the responses of the pollen of one series, nor was any comparison possible between similar slides of successive series.

Preceding the quantitative experiments it was necessary to determine the optimal concentrations of the test substances. Table 1 shows measurements made from cultures of snapdragon pollen with various concentrations of 3-indole acetic acid and vitamin B₁ at 25°C. The same tests were made with *Bryophyllum* and similar results were obtained. In both cases, concentrations of 3-indole acetic acid stronger than 1:50,000 were toxic, i.e., caused decreased germination, retarded elongation, and caused bursting and distortion of tubes. Weaker concentrations were favorably stimulative with a maximum response in the 1:100,000 dilution. Consequently, this dilution was used in the present studies.

By similar methods, vitamin B₁ was found to be toxic in a concentration of 1:10,000 but was with-

long to be measured accurately. With a growth period of 5½ hours for snapdragon pollen at the optimum temperature there were relatively few tubes longer than the diameter of the low power microscopic field. With *Bryophyllum* a three-hour culture period was sufficient. Growth was stopped by removing the slides from the germination chamber and placing them on a warming plate (50°C.). In this way, the excess moisture was removed in three to five minutes and the slides were ready for mounting in an aceto-carmine mounting medium (Zirkle, 1937). Slides prepared in this way were especially satisfactory for study because there was no disturbance of the tubes from the original growth pattern, and there was no loss of tubes or ungerminated grains, as usually results when a dip staining method is used.

For experimentation with different temperatures the germination pans were placed in constant temperature chambers, and the temperatures were adjusted so that the readings at the locus of the slides were 15°C., 20°C., 25°C., 30°C. and 35°C., respectively. The temperatures were controlled to within ±0.5°C. Therefore, with each pan holding 60 slides and with five temperatures being used, a total of 300 slides was used for each run. Each test substance was represented ten-fold at each temperature with each run. The runs were repeated numerous times.

For counts of percentages and measurements of tubes the low power field (100×) of a compound mi-

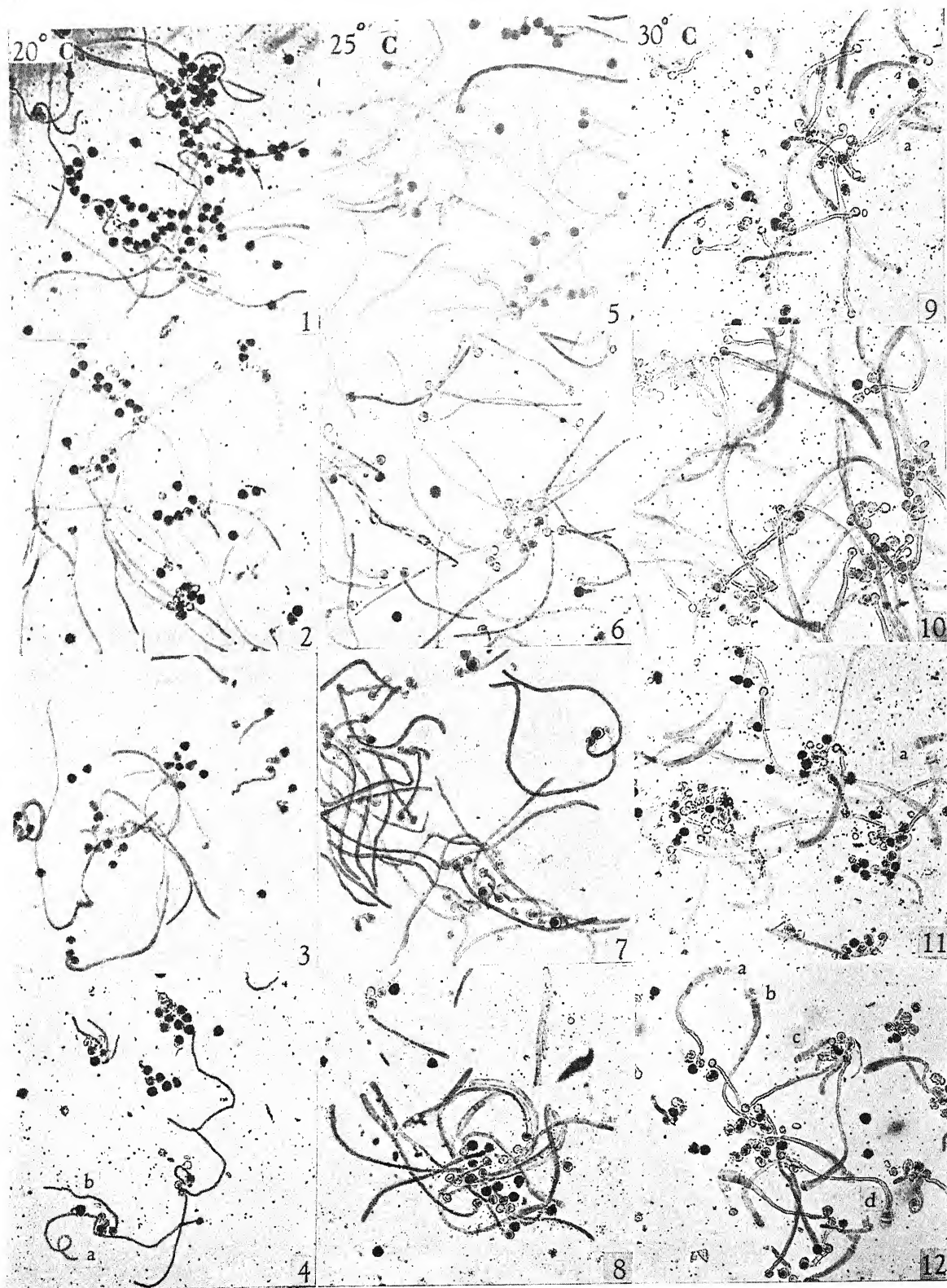


Fig. 1-12. Snapdragon. Each vertical column shows cultures grown at the same temperature in different media; horizontal rows represent growth in the same culture media at different temperatures.—Fig. 1, 5 and 9. Control.—Fig. 2, 6

TABLE 2. *Snapdragon*. 20°C. 5½-hour culture. For key to the abbreviations and numbers used in tables 2-7 see under table 2.

Series	pH	t.n.	n.g.	p.g.	t.w.	m.l.		0-1	1-2	2-3	3-4	4-5	5-6
Control	6.5	780	212	27.2	10.5	317	no.	23	69	75	38	7	0
							%	10.8	32.4	35.4	17.9	3.3	0
3-indole butyric	6.35	755	260	34.4	9.6	361	no.	23	66	81	69	18	3
							%	8.8	25.4	31.1	26.5	6.9	1.1
3-indole acetic	6.35	690	249	36.0	9.5	383	no.	22	53	71	76	21	6
							%	8.8	21.3	28.5	30.5	8.4	2.4
Naphthalene acetic.....	6.35	820	254	31.0	9.5	331	no.	25	78	87	53	10	1
							%	9.8	30.7	34.2	20.9	3.9	0.4
Vitamin B ₁	5.6	760	211	27.8	10.9	306	no.	24	74	76	31	6	0
							%	11.4	35.1	36.0	14.7	2.4	0
0.01% colchicine	6.6	909	200	22.0	10.5	258	no.	36	86	62	16	0	0
							%	18.0	43.0	31.0	8.0	0	0

Key to the abbreviations and numbers used in tables 2-7.

pH—Hydrogen-ion concentration.

t.n.—Total number of grains counted.

n.g.—Number of grains germinated.

p.g.—Percentage of germination.

t.w.—Mean tube width in microns.

m.l.—Mean tube length in microns.

Classes of tube lengths:

0-1— 0- 144 microns.

1-2—145- 288 microns.

2-3—289- 432 microns.

3-4—433- 576 microns.

4-5—577- 720 microns.

5-6—721- 864 microns.

6-7—865-1,008 microns.

7- 8—1,009-1,152 microns.

8- 9—1,153-1,296 microns.

9-10—1,297-1,440 microns.

10-11—1,441-1,584 microns.

11-12—1,585-1,728 microns.

12-13—1,729-1,872 microns.

croscope was used. Fields for study were chosen at random among those containing approximately 50 grains. This rule was instituted for purposes of producing uniformity, since it eliminated the counting of (1) very thickly populated fields, where germination and growth were affected by crowding, and (2) those fields containing only a very few grains, where growth was less uniform. (Further mention of the desirability of a uniform distribution of grains is made in the discussion of the auxin series.) Pollen of the two species used is sufficiently fine so that more

than 1,000 grains could be counted in one field. The measurements were made with an ocular micrometer, using a low power lens for length measurements and an oil immersion lens (990X) for measurement of tube widths. Since the diameters of the tubes were often not uniform, only the greatest diameter of each tube was measured.

The pH of each solution was determined at the time of inoculation. This was done colorimetrically with occasional checks by the calomel electrode potentiometer.

TABLE 3. *Snapdragon*. 25°C. 5½-hour culture.

Series	pH	t.n.	n.g.	p.g.	t.w.	m.l.		0-1	1-2	2-3	3-4	4-5	5-6	6-7
Control	6.5	722	426	59.0	14.5	357	no.	44	112	128	98	40	4	0
							%	10.3	26.3	30.0	23.0	9.4	0.9	0
3-indole butyric ...	6.35	708	460	64.9	13.2	410	no.	16	92	140	160	36	12	4
							%	3.5	20.0	30.0	35.0	7.8	2.6	0.9
3-indole acetic.....	6.35	725	480	66.2	13.0	454	no.	12	84	124	136	96	20	8
							%	2.5	17.5	25.8	28.3	20.0	4.2	1.7
Naphthalene acetic	6.35	720	438	61.0	13.1	411	no.	24	100	112	112	80	10	0
							%	5.5	22.8	25.6	25.6	18.3	2.3	0
Vitamin B ₁	5.6	718	431	60.8	15.0	340	no.	50	136	108	100	36	1	0
							%	11.6	31.5	25.1	23.2	8.3	0.2	0
0.01% colchicine...	6.6	847	432	51.0	21.0	298	no.	45	183	129	63	12	0	0
							%	10.4	42.4	29.9	14.6	2.8	0	0

and 10. Three-indole acetic acid.—Fig. 3, 7 and 11. Vitamin B₁.—Fig. 4, 8 and 12. Colchicine. Note the ungerminated grains in the 20°C. column; the bloating in the 30°C. column.—Fig. 4. a, Coiling of tube in colchicine series; b, an extremely rough tube which is characteristic of colchicine stimulation at low temperatures.—Fig. 9. a, Striation within tube.—Fig. 11. a, Striation in vitamin B₁ series.—Fig. 12. a, b, Exuded cytoplasm from the bloated tubes of the colchicine series; c, d, striations within tubes. Magnification approximately 77X.

TABLE 4. *Snapdragon*. 30°C. 5½-hour culture.

Series	pH	t.n.	n.g.	p.g.	t.w.	m.l.		1-2	2-3	3-4	4-5	5-6	6-7
Control	6.5	570	267	46.8	30.7	254	no.	52	102	103	10	0	0
							%	19.5	38.2	38.6	3.7	0	0
3-indole butyric ...	6.35	490	256	52.4	27.3	334	no.	24	70	100	54	6	2
							%	9.4	27.3	39.1	21.1	2.3	0.8
3-indole acetic.....	6.35	538	292	54.3	28.4	354	no.	30	48	132	70	10	2
							%	10.3	16.4	45.2	23.9	3.4	0.7
Naphthalene acetic	6.35	560	274	48.9	28.0	330	no.	38	90	110	53	3	0
							%	13.9	32.9	40.2	12.0	1.1	0
Vitamin B ₁	5.6	644	286	44.4	31.8	262	no.	48	124	90	22	2	0
							%	16.8	43.4	31.5	7.7	0.7	0
0.01% colchicine...	6.6	773	294	38.0	33.0	221	no.	76	140	70	8	0	0
							%	25.8	47.6	23.8	2.7	0	0

RESULTS AND DISCUSSION.—Less than 1 per cent germination occurred at 15°C. in all cases with pollen of snapdragon, and the tubes were extremely short and narrow. The only discernible response at this temperature was that germination occurred less frequently in the colchicine medium than in the others. Because germination was so slight and the resulting tubes were rarely more than twice the diameter of the grains in length, no counts or measurements are presented.

The results for the pollen of snapdragon are presented in tabular form for the 20°C., 25°C. and 30°C. series (tables 2, 3 and 4). Also, figures 1 to 12 illustrate some typical responses for these series. The 35°C. series showed virtually complete bursting of the tubes which rendered the task of counting and measuring a hopeless one. It was perceptible, however, that the character of the tubes resembled the corresponding series at 30°C. except that the tubes were shorter and the percentage of germination was less.

The observed responses of this pollen to temperature were the percentage of germination and the length, shape, and amount of bursting of tubes. At 20°C. the mean germination was 27.5 per cent in the control series as compared to 59.0 per cent at 25°C. and 46.8 per cent at 30°C. These data indicate that

25°C. is more favorable for germination than 20°C. or 30°C. Likewise, the lengths of tubes were found to attain their maxima at 25°C. Some of the tubes at that temperature were at least 144 microns longer than the longest of the 20°C. series and at least 288 microns longer than the longest of the 30°C. series.

The difference in the shape of tubes at different temperatures was striking. At 15°C. the tubes were very short, extremely narrow, and uniform in size throughout their length. At 20°C. and 25°C. (fig. 1 and 5) the tubes were broader but still uniform in width. At 30°C. an altogether different response was found. Here the intine penetrated the exine in a constricted form and then gradually enlarged or "battered" so that it became a club-shaped structure with the diameter of the distal part of the tube usually exceeding the diameter of the grain itself (fig. 9). At 35°C. the same characteristic bloating occurred, except that the swollen tubes nearly always burst, thereby allowing the cytoplasm to escape. These characteristics of increased temperatures (decreased germination, shortening and broadening of tubes, and high percentages of bursting) were found to exist with the other species studied, although the magnitude of swelling and the temperatures at which the responses occurred were specific for each. *Bryophyllum*, for example, showed little difference at

Table 5. *Bryophyllum*. 15°C. 3-hour culture.

Series	pH	t.n.	n.g.	p.g.	t.w.	m.l.		0-1	1-2	2-3	3-4	4-5
Control	6.5	1,056	304	28.8	4.6	183	no.	110	158	32	4	0
							%	36.2	51.9	10.5	1.3	0
3-indole butyric	6.35	869	357	41.1	3.5	263	no.	69	137	123	21	7
							%	19.3	38.4	34.5	3.9	1.9
3-indole acetic	6.35	948	375	39.5	3.5	264	no.	80	146	102	38	9
							%	21.3	38.9	27.2	10.1	2.4
Naphthalene acetic	6.35	1,015	364	35.8	3.4	235	no.	89	168	81	23	3
							%	24.5	46.2	22.2	6.3	0.8
Vitamin B ₁	5.6	1,390	365	26.3	5.6	187	no.	135	176	47	7	0
							%	37.5	48.2	12.9	1.9	0
0.01% colchicine	6.6	1,795	395	22.0	5.0	170	no.	139	244	12	0	0
							%	35.2	61.7	3.0	0	0



30°C. from 25°C., but at 35°C. the responses to increased temperature began to appear. Various workers (Buchholz and Blakeslee, 1927; Dorsey, 1919; Anthony and Harlan, 1920, and others) have studied the relation of temperature to the rate of pollen tube elongation but none mentions the increase in diameter with increased temperature. Vom Berg (1929), working with *Impatiens Holstii*, reported that 32°C. was "less favorable" for germination and elongation than 20°C. His test temperatures were 10°C., 20°C. and 32°C.

In all cultures of snapdragon at 30°C. there was a peculiar response within the swollen portion of the tube. Quite frequently cross striations appeared which were apparently cytoplasmic material and not callose plugs (see fig. 9a, 11a, 12c). This response was not found with any other species.

In consideration of tube width, percentage of germination and the occurrence or absence of bursting, 25°C. was more favorable than the other temperatures for the growth of the pollen of snapdragon.

The response to stimulation by auxin, as with the response to temperature change, is also an individual reaction of the species concerned. Pollen is naturally furnished with auxin in greater or lesser amounts, and if the natural supply is adequate the provision of additional auxin should cause little, if any, stimulation. The writer (Smith, 1939) found that 3-indole acetic acid induced the pollen of *Pinus Austriaca* to germinate, whereas germination did not occur in the control. The percentages of germination and the rates of elongation of the tubes of the pollen of other species were also reported. Cooper (1939) and others have noted mild stimulation of germination by the auxins. In the present study, with snapdragon, the auxins showed themselves to be favorable additions to the culture medium, although the response was less pronounced than with *Bryophyllum*. With each auxin and at each temperature, longer tubes, narrower tubes and higher percentages of germination were found than in the corresponding control (see tables 2, 3 and 4). The frequency of the tubes in the various length-classes is noteworthy. In each case the percentage of short tubes (the 0-1 and 1-2 groups) was less than in the control. There was a general stimulating effect so that, although the germination was not greatly increased, the tubes elongated more rapidly and attained a greater length than did those of the control.

Chemotropic effect of stigmas and other flower parts on the growth of pollen was observed by Molisch (1893). Subsequent investigations have shown many cases of similar responses, although they are

not universal. That growth of tubes is enhanced by clustering of the grains was noted by Brink (1924b). This was observed with many species, and none of those tried failed to respond in this way. He reports, furthermore, that germination is quite frequently much more profuse when the grains are grouped closely together. In the present work, the same phenomenon was observed with the pollen of snapdragon and *Bryophyllum*. In the control, the vitamin B₁ and the colchicine series, the tubes from grains that occurred in clusters had a marked tendency to grow into the cluster. If a tube of this cluster-arrangement happened to be pointed outward by chance, it usually bent inward and grew back into the cluster. Germination was also usually enhanced by the grouping of grains. Since the concentration of auxin can reasonably be expected to be greater in regions thickly populated with grains, it is apparent that tubes are stimulated to grow toward the auxin center just as Molisch (1893) observed them to grow toward stigmatic tissue.

The tubes of the auxin series were more nearly straight, with less tendency to coil and bend in their outward growth, than were the control tubes. A common response was for the tubes to radiate away from the cluster. Inasmuch as the medium had been supplied with an external supply of auxin, the tubes were evenly stimulated and grew in the direction in which they started. This results in growth away from the cluster even though the tube may have to go through the cluster and emerge on the other side, should it be pointed toward the cluster when germinating. Under such conditions, unilateral hormonal stimulation from other grains and tubes was overshadowed. Such a result was seen many times.

Of the three auxins, 3-indole acetic acid seemed to have a stronger stimulatory effect on snapdragon pollen than the 3-indole butyric or naphthalene acetic acid. The latter substance showed the least stimulatory effect.

No evidence of favorable stimulation by vitamin B₁ was revealed in these experiments. Dandliker, Cooper and Traub (1938) state that in their work with pollen of *Carica papaya* the influence of vitamin B₁ was one of accelerating the rate of early tube growth, although in certain varieties of this pollen they found no stimulation. In the present investigation, no attempt was made to study the early growth of tubes, but the results show that the addition of vitamin B₁ had little if any effect upon the final percentage of germination and length of tubes. The pH of this medium was 5.6, which was considerably more acidic than the control's 6.5, and may well be respon-

Fig. 13-24. *Bryophyllum*. Again each column represents one temperature and each row represents one culture medium.—Fig. 13, 17 and 21. Control.—Fig. 14, 18 and 22. Three-indole acetic acid series.—Fig. 15, 19 and 23. Vitamin B₁.—Fig. 16, 20 and 24. Colchicine. Note the broadening effect in each series with each temperature increase. The long tubes of this pollen show a broadening of the basal part of the tubes while the short tubes show a uniformly large diameter in the 35°C. cultures. In figure 18, note the strikingly long, narrow tubes of the 3-indole acetic acid series as compared to the other members of this temperature series (17, 19 and 20).—Fig. 20 is of the 0.1 per cent colchicine series and shows the extreme amount of bursting found with this concentration.—Fig. 16 and 24 are 0.01 per cent colchicine cultures. The similarity of the tubes of the control series and the vitamin B₁ series is quite evident. Magnification approximately 77X.

sible for the small difference. A slightly greater tube diameter than that of the control cultures was the only obvious difference. That this difference in diameter may well have been due to the pH change accompanying the addition of the vitamin (thiamin hydrochloride) was indicated by acidifying some of the control culture solutions to 5.6 with HCl. In this medium there was a similar increase in tube diameter.

With the colchicine series, in all three temperatures, the percentages of germination were decidedly less than those in the control. Inspection of tables 2, 3 and 4 reveals that, in each case, the length-class with the largest frequency is one unit less than the corresponding class in the control. This is a reflection of the fact that colchicine depresses germination and tube elongation, producing a larger population in the shorter categories. Figure 4 shows an interesting and characteristic colchicine reaction at low temperatures, that is, the coiling of the tube (a). Also, rough and crooked tubes (b) are common in the 20°C. cultures containing colchicine. At 15°C. and 20°C. no noticeable increase in tube width was perceptible. At 25°C. many of the tubes showed abnormal swelling or bloating which resembled the response of all the other series at 30°C. At 30°C. the bloating effect was magnified, and the swollen tubes frequently broke at the tips and the cytoplasm was forced out (fig. 12 a, b). Bursting of the tubes of the other 30°C. series was rarely found. Eigsti (1940b) observed that swollen tubes resulted from colchicine treatment of *Polygonatum*.

Pollen of *Bryophyllum* was found to be less sensitive to temperature changes than was that of snapdragon. As a result, only the 15°C., 25°C. and 35°C. cultures were counted and measured (tables 5, 6 and 7). Photomicrographs of the same four series presented for snapdragon are shown, the microscopic fields representing a random sample of the particular group (fig. 13 to 24).

At 15°C. 28.8 per cent germination and good growth of *Bryophyllum* pollen occurred, and the three-hour culture period, necessary to produce maximum development at higher temperatures, was sufficiently long to allow considerable growth at this lower temperature. At 25°C. 61.5 per cent germination and at 35°C. 43.7 per cent germination resulted. Cultures were tried at 45°C. but gave virtually complete bursting of both grains and tubes. The tubes of this pollen were rather smooth and inclined to moderate curvature in their growth patterns. Probably the most striking response to the increase in temperature, in addition to the greater mean tube width, was the very uneven and irregular growth at 35°C. In both the 15°C. and 25°C. series the tubes were more or less uniform in length with few unusually short or particularly long tubes. At 35°C., however, there was a very diverse mixture of tube lengths from very short to quite long (fig. 21). The longest tubes observed in the controls were in this series and were at least 288 microns longer than the longest in the 25°C. series. The short tubes of this group had a greater diameter than the long ones (again see fig. 21). Also,

the longer tubes were broader in the region nearest the grain and gradually tapered out at the extremity. Thus, although the tubes of both *Bryophyllum* and snapdragon exhibited a broadening response to increased temperature, the methods of response were opposite. Snapdragon produced tubes that were nearly isodiametric at 20°C. and 25°C. and then responded with a great broadening at the outer extremities of the tubes at 30°C. *Bryophyllum*, although isodiametric at 15°C., began to show an unevenly increased diameter at 25°C. and a still greater enlargement at 35°C. The broadening in this case was at the proximal end of the tube (compare fig. 13, 17 and 21).

As has been noted above, pollen of *Bryophyllum* gave a more marked reaction to the addition of auxin to the medium than did that of snapdragon. The percentages of germination were greater in the presence of auxin and the mean lengths of the tubes were considerably greater. Again 3-indole acetic acid was apparently more stimulating than naphthalene acetic acid but had an effect about equal to that of 3-indole butyric acid. It is evident that the greatest stimulation occurs at the temperature (25°C.) that evoked the optimum growth in the control. Here 85 per cent germination was found in the 3-indole acetic acid series and a mean tube length of 562 microns, in contrast with 61.5 per cent and 334 microns in the control. Visible evidence of increased germination may be seen in figure 18 where relatively few ungerminated grains appear as compared to figure 17. A third response to the auxins (besides increased germination and tube length) was the relatively smaller tube width at all temperatures than in the corresponding control. Accompanying a smaller tube width is the fact that the tubes were again relatively straighter in the auxin series. The tendency for a great mixture of tube lengths at 35°C., as mentioned in the case of the control series, existed in the auxins as well as in the vitamin B₁ and colchicine series. Indeed, the longest tubes found with *Bryophyllum* pollen were in 35°C. 3-indole acetic and 3-indole butyric acid series, where a few tubes attained a length of over 1,700 microns in the three-hour culture period. The predominance of shorter tubes, however, cut down the mean length to less than that of the 25°C. group.

As with snapdragon, little difference between the vitamin B₁ series and the control was noticeable. The percentages of germination, the mean lengths of the tubes and the general character of the tubes of the two series were very much alike. A consistently greater tube diameter was again the only prominent response. The similarity of the two series is seen in a comparison of figures 17 and 19.

In the colchicine series of *Bryophyllum* pollen, three concentrations of colchicine were used in the 25°C. and 35°C. tests. At 15°C. the 0.01 per cent (1:10,000) concentration caused almost 7 per cent decrease in germination, a slightly greater mean diameter and extreme crookedness of the tubes (fig. 16). At 25°C. these same general reactions were more pronounced with an increase in the concen-

tration of colchicine. The 0.1 per cent (1:1,000) concentration greatly reduced germination, retarded growth and caused extensive bursting of tubes (see fig. 20). The 0.005 per cent (1:20,000) concentration showed responses that were very similar to the control series with only a slight slowing of growth and a slight increase in the crookedness of the tubes. The 0.01 per cent revealed marked inhibition of germination and retardation of tube growth, whereas the 0.1 per cent was extremely toxic.

At 35°C. almost complete bursting of tubes resulted in the 0.1 per cent series, and much bursting was found also in the 0.01 per cent group. The 0.005 per cent series was, again, essentially like the control. In the higher concentrations the tubes showed the characteristic shortness and crookedness that colchicine was invariably observed to evoke in this pollen. Because no bursting of tubes occurred at 15°C. or 20°C., it is evident that the effect of the drug is somewhat enhanced by the higher temperature.

In order to determine whether colchicine might show favorable stimulatory effects at a higher dilution, a series of pollen was grown in 0.001 per cent (1:100,000) colchicine at 25°C. Measurements of this series revealed no departures from the control.

A recent paper by Beck and Joly (1941) mentions some points which are in general accord with findings noted in the present paper, as well as with those in a previous report (Smith, 1939). These are as follows: (1) that "old" pollen shows less germination than fresh pollen; (2) that 3-indole acetic acid increases the germinative power of pollen; (3) that germination is enhanced by clumping of the grains and (4) that tubes have a tendency to grow outward and away from the cluster if artificial auxin is supplied to the growth medium.

Beck and Joly report bursting of tubes following enlargement of the tips of the tubes of the monocotyledonous pollen with which they work. Such a reaction was not observed at ordinary temperatures with any of the species used in the present study. In cultures of *Bryophyllum* and sweet pea which were buffered and adjusted to a pH scale extending from pH 3.5 to 9.5, however, a very high percentage of the tubes in the 3.5 and 9.5 cultures ceased elongation after attaining a length of about 50 microns and burst following the swelling of their tips into spheres. Inasmuch as iso-electric gelatin was used as a buffer and HCl and NH₄OH as adjusters, it seems probable that the swelling and bursting was due to the unfavorable pH and not to the toxicity of these substances.

SUMMARY

Studies of the germination of pollen of *Antirrhinum majus* and *Bryophyllum Daegremontianum* were designed to show the effects of the addition of 3-indole acetic acid, 3-indole butyric acid, naphthalene acetic acid, vitamin B₁ and colchicine to the culture medium. The effects of different temperatures on these cultures were investigated by the sugar-agar technique.

The use of pollen from a single flower for the inoculation of the cultures of the various test substances was found to be essential to studies of the pollen of these species. This procedure eliminated the variability resulting from differences in the physiological age of the pollen of different flowers.

The pollen of all species studied showed an increase in diameter of the tubes corresponding to an increase in the temperature of the environment. Snapdragon pollen showed (1) negligible growth at 15°C., (2) optimum germination and length of tubes at 25°C., (3) pronounced broadening and bloating of the distal portions of the tubes at 30°C., and (4) extensive bursting of the bloated tubes at 35°C. *Bryophyllum* pollen showed (1) marked growth at 15°C. and (2) optimum germination and length of tubes at 25°C. At 35°C. a great diversity of tube lengths resulted with many short, broad tubes and some extremely long ones with broadened proximal portions.

The auxins were favorable additions to the culture medium with only moderate stimulation in the case of snapdragon, whereas much greater stimulation of both germination and tube elongation was obtained in *Bryophyllum*. The greatest auxin stimulation occurred at 25°C., which was also the optimum temperature for growth of the pollen of these species. Three-indole acetic and 3-indole butyric acids were more effective stimulants than naphthalene acetic acid. Concentrations of auxins weaker than 1:50,000 were favorably stimulative. All stronger concentrations were toxic as indicated by decreased germination, bursting and distortion of tubes.

Evidence of favorable stimulation by the addition of vitamin B₁ was not found. The slightly greater tube width occurring in this medium is attributed to the increase in acidity resulting from the addition of the vitamin.

Colchicine was depressive to both germination and tube elongation at all temperatures used. In a highly dilute solution (1:100,000) the growth responses approached the condition found in the control. At temperatures of 25°C. and above, broadening and rupturing of the tubes were much more prominent than in the control series. Below 25°C. no bursting of the tubes was found.

The pollen of snapdragon manifests the peculiar response of bloating of the tubes to the following factors which are unfavorable to its growth: (1) temperatures of 30°C. or more, (2) the presence of colchicine in the culture medium, (3) high acidity and (4) "old" pollen, i.e., pollen from flowers that have dehisced several hours previous to inoculation.

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ACCUMULATION OF NICOTINE IN RECIPROCAL GRAFTS OF TOMATO AND TOBACCO ¹

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SINCE THE discovery of the general nature of nicotine by Pinner (1893) considerable interest has centered about the origin and the physiological functions of this alkaloid in the tobacco plant. The problem at present, however, is far from a satisfactory solution, principally because of the failure of the usual methods of experimental manipulation to influence the nicotine synthetic mechanism in a consistent manner. Indeed, Mothes (1928) has commented upon both the extreme stability of nicotine metabolism in the plant and the great difficulty to be encountered in any attempt to alter its course.

Heretofore, investigations dealing with the origin of nicotine appear to have been predicated almost entirely upon the assumption that the tobacco leaf is the principal seat of nicotine formation. The common observation that the leaf usually contains a greater proportion of nicotine than either root or stem provides a reasonable basis for such a view. An examination of the literature, however, has revealed facts which seem to be irreconcilable with this view. In the first place, the nicotine content of tobacco leaves appears to bear no constant relationship to size, growth, or to any other obvious characteristic, either morphogenetic or physiological, of the leaf (Mothes, 1928). Secondly, although tobacco leaves and shoots in various stages of development have been removed from the roots and cultured under a wide variety of conditions (Vickery, *et al.*, 1937; Mothes, 1928; Dawson, 1940), only in isolated and rather unusual cases has there been an apparent increase in the nicotine content of the detached aerial parts of the plant. It should, perhaps, be remarked in this connection that leaves and shoots in various

stages of development have been employed in these experiments with the result that the nicotine synthesizing capacity of young growing tissues as well as of fully matured tissues appears to have been rather fully explored.²

An obvious implication of these findings is that nicotine may not be produced in the aerial portions of the plant at all. Rather, if some connection between root and shoot is necessary for the continued accumulation of the alkaloid in stems and leaves, the possibility must be considered that nicotine (or its immediate progenitor) may be produced largely or perhaps solely in the roots. The present study was designed to follow the accumulation of nicotine in the aerial portions of tobacco plants when they are cultured outside the sphere of influence of tobacco roots and to investigate the ability of tobacco roots to donate nicotine to the aerial portions of another species of plant to which they may be grafted.

METHODS.—Plants of *Nicotiana tabacum* L. var. Turkish and of *Lycopersicum esculentum* Mill. var. John Baer were grown from seed in pots of white quartz sand to which were added daily applications of a mineral nutrient solution (Shive and Robbins, 1938). When the stems of both plants had reached a diameter of approximately three-eighths of an inch at the base they were severed at a point twelve inches from the roots and split to receive the scions. From the tobacco shoots thus removed the terminal four inches including the apical meristem were excised to serve as scions upon the tomato stocks. The tomato scions were secured from the older and more stocky

² Attempts in this laboratory to obtain nicotine from the lateral shoots which form on tobacco stalks during culture in moist chambers have been fruitless. Likewise, it has been impossible to detect nicotine in the callous tissue which forms over the basal ends of tobacco stalk segments during culture under the same conditions.

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portions of the stems and contained one lateral bud each. The majority of the graft unions were of the cleft type, but a few approach grafts were also made. One-half of the tobacco and tomato stocks were stripped of their leaves at the beginning of the experiment. These are designated herein as leafy and leafless stocks, respectively.

The samples of leaf, stem, and fruit tissue were ground through a food chopper and then extracted with boiling water which had been acidified with dilute sulphuric acid. Nicotine was determined upon aliquots of these extracts by a method which has been described elsewhere (Dawson, 1939). In this method the total volatile bases including nicotine are removed from triplicate samples by simultaneous steam distillation. The distillates after titration are redistilled under reduced pressure at 40° C. By this procedure ammonia and the low-boiling amines are quantitatively removed and estimated by titration. Nicotine is calculated by the difference between the two titrations. Nicotine was isolated from some of the extracts as the dipicrate in order to furnish qualitative evidence for the presence of the alkaloid.

Because of the impracticability of performing large numbers of grafting operations the size of the samples selected for analysis was necessarily small (usually four plants). In some measure this objection has been met by collecting several such samples at intervals over a considerable period of time. However, the quantitative aspect of the data can assume only approximate significance, and the qualitative relationships are to be emphasized.

The data included in this paper represent the results of two separate experiments which were conducted during the early spring and the summer of

1941. The second experiment as regards both methods and results was essentially a duplication of the first, although larger numbers of grafts were prepared and only one sample was taken for analysis.

EXPERIMENTAL RESULTS.—Nicotine in tobacco scions upon tomato stocks.—The average nicotine content of one original tobacco scion at the time of preparation of the grafts was 1.3 mgm. (table 1). After thirty-one days of development upon tomato stocks, at which time increase in length of stems had been threefold and a total of ten new leaves had been produced upon each scion, the nicotine content per scion was 3 mgm. At the end of another twenty-eight days the scions contained an average of thirty-eight leaves each and had reached a stem length of approximately thirty inches. At this time the nicotine content per scion was only 4.1 mgm. Such slight increases are somewhere near the order of magnitude of experimental error and may be considered as indicating no change at all in total nicotine content until such time as the validity of the apparent change may become established by further experimentation.

Subdivision of the third collection of tobacco scions into three parts yielded some evidence concerning the fate of the nicotine in the original scion. The basal ten to eleven inches of each scion contained the entire quantity of nicotine (4.1 mgm.) which had been present in the beginning. The central portions of the scions up to the inflorescences contained no traces of nicotine, although this fraction represented 57 per cent of the sample in terms of fresh weight. The inflorescence of each scion, which contained buds, flowers, and pods in nearly all stages of maturity, was found to possess an average high-boiling base content of 1.3 mgm. (calculated as nicotine).

TABLE 1. Nicotine content and fresh weights of reciprocal grafts of tobacco and tomato. The values for fresh weight and nicotine content are expressed in terms of the average number of grams or of milligrams in one individual stock, scion, or fruit.

Sample	March 11		April 11	May 9		June 3	
	Weight gm.	Nicotine mgm.	Nicotine mgm.	Weight gm.	Nicotine mgm.	Weight gm.	Nicotine mgm.
Tobacco scions on leafless tomato stocks.....	3.7	1.3	3.0
Tobacco scions on leafy tomato stocks.....	3.7	1.3	2.6	149.0	4.1	436.2	8.6
Lower leaves	55.3 ^a	4.1	22.0	2.5
Rest of leaves	74.9 ^a	0.0	119.2	0.0
Stems	145.0	6.1
Inflorescences	18.8	1.3 ^b	150.0	17.6 ^b
Tomato stocks	0.0
Tomato scions on leafless tobacco stocks....	...	0.0	37.6	99.4	134.9	364.0	500.7
Ripe fruit	60.8	1.3
Green fruit	41.3	0.6
Tomato scions on leafy tobacco stocks.....	...	0.0	55.5	213.2	258.3	372.3	570.5
Lower leaves	23.0	97.8
Remainder of leaves	94.2	148.9
Stems	96.0	10.0
Fruits	7.6	1.6
Ripe	48.6	3.5
Green	441.1	1.5
Leaves of tobacco stocks.....	...	23.6	296.5	74.5	229.9	55.0	194.0

^a These values include stems as well as leaves.

^b Values not included in the calculation of the total nicotine content of the scions due to failure of the picrates to behave as nicotine dipicrate.

Positive identification of this volatile fraction as nicotine, however, was not made. It seems possible that this minute amount of material may have been composed of other high-boiling amines than nicotine (*cf.* Klein and Steiner, 1928).

Twenty-five days after the preceding collection a fourth sample of four plants was obtained, which presented a slightly different distribution of nicotine. The lower six leaves from each scion contained only 2.5 mgm. of nicotine, while none at all was found in the remaining forty-two leaves. In each stem, however, 6.1 mgm. of nicotine were found. The analytical data indicated the presence in the inflorescences of 17.6 mgm. of a high-boiling base which did not appear to be nicotine, since attempts to prepare the pieric acid derivative yielded only a yellow oil which could not be induced to crystallize.

The presence or absence of leaves on the tomato stocks did not seem to affect the total nicotine content of the tobacco scions (table 1). The rates of formation of the graft unions and subsequent growth of the tobacco scions, however, were appreciably increased in their presence. Analyses performed upon the tissues of the tomato stocks indicated that no nicotine had been translocated out of the scions.

The data from the second set of experiments are found in table 2. It will be noted that a 24-fold increase in the fresh weight of one tobacco scion during its development upon tomato roots was accompanied by an apparent net increase of only 0.6 mgm. of nicotine, a quantity entirely incommensurate with that which would be expected to obtain in a normal tobacco plant.

TABLE 2. Nicotine content and fresh weights of reciprocal grafts of tobacco and tomato from second experiment. The values for fresh weight and nicotine content are expressed in terms of the average number of grams or milligrams in one individual scion or shoot.

Date	Sample	Fresh weight	Nicotine
		gm.	mgm.
June 9	Original tobacco scions (9 in sample)	1.23	1.73
July 14	Tobacco scions on tomato stocks (20 scions in sample)	30.3	2.3
	Tomato scions on leafless tobacco stocks (15 scions in sample)	11.8	15.2
	Tobacco shoots from plants which had been decapitated at the time of preparation of the grafts. (Ten shoots in sample)	29.2	21.2

From the evidence at hand it cannot be stated unequivocally that the tobacco scions did not manufacture at least small amounts of nicotine. Inspection of the values in table 1 reveals that the nicotine content of the tobacco scions upon tomato stocks increased slowly but regularly throughout the period of culture. If this synthesis was actual and not the

result of sampling error or of an error in the analyses, then it raises an interesting question as to the kind of cell which in the stem or leaf can synthesize the pyridine and pyrrolidine ring structures of nicotine. In any event, it is quite apparent that the tobacco scions did not synthesize appreciable quantities of the alkaloid.

Nicotine in tomato scions upon tobacco stocks.—The distribution of nicotine between stock and scion in grafts of this type was in certain respects the reverse of that previously discussed for the reciprocal type. In the beginning there was, of course, no nicotine present in the tomato scions. The leaves of each of the tobacco stocks contained an average of 24 mgm. of nicotine. At the end of thirty-one days (table 1) the average tomato scion on the leafless tobacco stock had grown to a height of approximately fourteen inches while similar scions on the leafy tobacco stocks had grown an average of twenty inches in length. Analyses for nicotine indicated the presence in the tomato scions on the leafless tobacco stocks of an average of 37.6 mgm. of nicotine per scion and in the tomato scions on the leafy tobacco stocks of an average of 55.5 mgm. of nicotine per scion. The identity of the nicotine present in these tomato tissues was established by direct isolation.³ At the end of fifty-nine days of growth the tomato scions on the leafy and leafless tobacco stocks contained 258.3 and 135 mgm. of nicotine,⁴ respectively.

The influence of the leafy stocks was evident in hastening the development of the graft union and subsequent growth of the scion, so that the increased nicotine content of these scions compared with that of the scions on the leafless stocks is not to be considered unusual.

Changes in the nicotine content of the tobacco leaves on the leafy tobacco stocks were also of interest. In thirty-one days the quantity of nicotine present in these leaves had increased from 24 to 297 mgm. Such a change is to be expected as a result of the decapitation of the plant (Garner, *et al.*, 1934). The decreases to 230 mgm. and to 194 mgm. which occurred at the end of fifty-nine days and of eighty-four days, respectively, present a slightly different problem. If this decrease represents a loss by translocation or by volatilization, then such results are in conflict with those already discussed in connection with the fate of the nicotine in the tobacco scions on tomato stocks. It is necessary to note, however, that the leaves involved in this sample were all basal leaves most of which were so old that the chlorophyll had disappeared from the areas between the veins, and browning was evident along the leaf edges. A loss of nicotine from these leaves, therefore, may have represented simply a normal consequence of senescence (Vickery, *et al.*, 1935).

³ Eighty mgm. of nicotine dipicrate were isolated from 75 mls. of the hot water extract, a yield of 62 per cent of the theoretical. The crystals after recrystallization from boiling water decomposed at 224–225°C. (corr.). A mixture of these crystals with those of pure nicotine dipicrate yielded no depression in the decomposition point.

⁴ Decomposition point of the isolated dipicrate derivative was 224–225°C. (corr.).

The stems of the scions contained an average of 10 mgm. each of nicotine. Compared with the relative distribution of nicotine in the shoots of field grown tobacco (Vickery, *et al.*, 1935) the distribution of the alkaloid in the tomato scions of this experiment is quite similar and suggests that the same fundamental processes of nicotine synthesis and accumulation may prevail in each case. If so, this would seem to provide ample justification for the use of graft hybrids in the present study.

Flowering in the tomato scions was profuse and a rather large number of fruits were developed. Interest attendant upon the possibility of the occurrence of nicotine in the fruits led to an examination of the macerated whole fruit tissues by the methods already described. Nine green fruits ranging in size from 4 to 30 mm. diameter were found by quantitative analysis to contain a total of 6.4 mgm. of nicotine, or an average of 0.7 mgm. of the alkaloid per fruit. In terms of percentage of fresh weight these figures represent a nicotine content of 0.02, a value which is close to the limits of error of the methods employed. In order to substantiate these results, therefore, 125 mls. of the 165 mls. total volume of the fruit extract were subjected to steam distillation, and the alkaline distillate extracted with ether. Upon evaporation of the ether the characteristic pungent odor of nicotine was detected, and when added to two mls. of a saturated aqueous solution of picric acid a definite precipitate of nicotine dipicrate was formed. This precipitate was filtered off on a micro funnel and recrystallized from two mls. of hot water. The crystals were thrown down with a semi-micro centrifuge, and the supernatant fluid removed with a glass capillary by slight suction. A drop of alcohol was added to remove the excess picric acid, the alcohol was removed by suction, and the crystals were dried. The decomposition point of the material so obtained was 223–224°C. (corr.). It appears, therefore, that some of the nicotine must have been translocated to the tomato fruits, although the order of magnitude of such transport was extremely small.

Twenty-five days after the preceding collection a fourth sample of four plants was obtained. The tomato scions were rather large and bore a substantial number of fruits. The individual scions from the leafless tobacco stocks carried 1.3 mgm. of nicotine in the one ripe fruit and 5.4 mgm. in the nine green ones. The leaves and stems contained 500.7 mgm. of the alkaloid. The aqueous extracts of these latter tissues were combined and exhaustively distilled with steam after the addition of sodium hydroxide to alkalinity. The distillate was evaporated and the residues extracted with ether. From the ether extract 4.70 grams of crude nicotine dipicrate⁵ were obtained, a yield of 93.4 per cent of the theoretical.

From the leafy tobacco stocks four tomato scions were removed at the same time and examined for nicotine. Each scion bore one ripe fruit which con-

tained on the average 1.5 mgm. of nicotine and ten green fruits which contained a total of 35 mgm. The leaves and stems of the scions contained an average of 571 mgm. each, while each tobacco stock had accumulated a total of 194 mgm. of the alkaloid in the leaves.

One observation of a qualitative nature appears to be of some interest. Soon after the development of the first leaves on the tomato scions the edges of the leaflets developed a dry brown necrosis which was sharply set off from the normal green tissue. Whether there was a causal relationship between nicotine and this injury is not known, but for reasons which are given below there seems to be a good possibility that such was the case.

In a repetitive experiment quantities of nicotine accumulated in the tomato scions which were approximately 70 per cent as great as the quantities which accumulated in the lateral shoots of tobacco plants which had been decapitated and defoliated at the time of preparation of the tobacco and tomato grafts. Examination of the data in table 2, however, indicates that a direct comparison between the amounts of nicotine which accumulated in the tomato scions and in the intact tobacco shoots at the same stage of development were not of much value. The reason for this appears to be that differences in leaf shape and area and differences in photosynthetic efficiency resulted in differences in the quantities of foodstuffs delivered to the roots, the control and estimation of which would have been quite difficult if not impossible. It has been obvious throughout these experiments that the production of nicotine by the roots is profoundly influenced by the photosynthetic capacity of the tops.

Nicotine in approach grafts of tomato upon tobacco.—All the foregoing data were obtained upon cleft grafts. Two plants consisting of tomato scions upon tobacco stocks were obtained by the method of approach grafting. In principle it would appear that the movement of materials across such a graft union would involve some degree of lateral transport. That this actually occurred is indicated by the following data. After sixty-six days of growth the two scions weighed 129 grams dry. Both scions bore at this time 120 gm. of fruit (fresh weight). The tomato scions contained 382 mgm. of nicotine each, while the leaves on each tobacco stock contained 36 mgm. of nicotine.

Downward movement of nicotine in tomato scions.—One plant of tomato was decapitated and the tip of the cut stem grafted into the stem of an intact tobacco plant. After the union had occurred, the tobacco stock was allowed to develop a lateral shoot with the idea in mind that some of the food material necessary for the development of the tomato shoot might be translocated down the tomato stock from the tobacco plant and thence up the tomato shoot. If such translocation did occur, nicotine did not appear to be among the materials so transported, since no traces of the alkaloid could be found in the leaves of the tomato stock or of the tomato shoot.

⁵ The once-recrystallized material softened at 221°C. and decomposed at 225°C. (corr.). A mixture of this preparation with a sample of pure nicotine dipicrate yielded no depression in the decomposition point.

TABLE 3. *Nicotine content and fresh weights of approach grafts of tomato and tobacco. Grafts prepared June 9, 1941, and harvested July 19, 1941. The values for fresh weight and for nicotine content are expressed in terms of the average number of grams or of milligrams in one individual "stock" or scion.*

Sample	Fresh weight	Nicotine
	gm.	mgm.
"Leafless" tobacco grafts:		
Tobacco "stock"	19.4	4.5
Tomato stem	99.0	7.8
Seven leaves from sector of tomato stem immediately above insertion of tobacco "stock"	39.5	69.0
Remainder of leaves on tomato stem (17.5 per plant)	88.0	13.2
"Leafy" tobacco grafts:		
Tobacco "stock"	62.0	192.8
Tomato stem	112.0	3.4
Ten tomato leaves from sector of tomato stem immediately above insertion of tobacco "stock"	26.4	15.8
Remainder of leaves on tomato stem (22 per plant)	102.0	10.0

Unilateral movement of nicotine in tomato scions.

—Eight tobacco plants were similarly decapitated and grafted into the sides of the stems of eight tomato plants. Four of the tobacco plants were defoliated. Union of the grafts occurred readily, and the tomatoes grew normally for some time. It was eventually noticed, however, that the same leaf injury, which had been noted in all cases in which nicotine had accumulated in tomato leaves, also occurred in grafts of this type. Certain aspects of the distribution of the injury, however, seemed significant. First, only the tomato plants which were grafted to the leafless tobacco "stocks" acquired the characteristic browning and necrosis of the leaflet edges. Those which were grafted to the leafy tobacco "stocks" were entirely free from injury. Secondly, only the leaves on that longitudinal sector of the stem which was directly above the graft union were damaged. The remainder of the leaves on the tomato stems were apparently perfectly normal.

The above behavior was correlated with similar changes in the nicotine content of the tomato leaves. In the leafless tobacco grafts 84 per cent of the total amount of nicotine in the leaves of all the tomato scions was found in the twenty-nine leaves which were located in the sector of the stems immediately above the points of insertion of the tobacco "stocks." The remaining 16 per cent of the alkaloid was found in those seventy tomato leaves which were located in all other sectors of the tomato stems. The tobacco stocks as well as the tomato stems contained only small amounts of the alkaloid. On the other hand, in the leafy tobacco grafts the great bulk of the nicotine was not located in the tomato scions at all but was found in the leaves of the tobacco "stocks." Of the quantity of nicotine which did find its way to the to-

mato scions in these grafts, approximately 61 per cent was located in the ten leaves in the stem sector immediately above the graft union, while the remainder was found in the remaining sixty-five leaves on all other sides of the stems (table 3).

Such data seem to provide an almost irrefutable argument for the translocatory origin of nicotine in the leaves of these graft hybrids. The path of transport is entirely unknown. Since movement seems to be upward in all cases and never down, it is tempting to suggest that the nicotine may be translocated in the xylem and that nicotine is not normally found in any other tissues of the stem in any appreciable quantities. Experiments are at present under way in which the nicotine content of the various tissues of the tobacco stem will be determined after they have been separated from one another by dissection. The accumulation of such large quantities of nicotine in the leaves of the tobacco "stocks" rather than in the leaves of the tomato "scions" in the latter type of graft may best be explained by assuming that the larger photosynthetic area exposed by the leafy as compared with the leafless "stocks" made it possible for the tobacco roots in the former case to synthesize much more nicotine than would otherwise have been possible. As the nicotine was then translocated up the stem it tended to accumulate mostly in the tobacco leaves rather than in the tomato "scion" which was probably supplied in large part by its own root system. The failure of the nicotine to induce visible injury in the tomato leaves above the graft union in the leafy tobacco grafts was obviously due (table 3) to its low concentration.

DISCUSSION.—From the foregoing it is clear that nicotine accumulates only in those graft hybrids which possess a tobacco root system. Furthermore, the alkaloid does not appear, in appreciable quantities at least, in graft hybrids, the tobacco component of which is the aerial shoot.⁶ The significance of these observations appears to lie principally in the fact that *nicotine (or its progenitor) was synthesized in the tobacco roots and translocated to the tomato leaves, and that it was not synthesized in appreciable quantities in the tobacco leaves.*

It has been possible to induce growth in tobacco leaves and stems without either the presence or the resulting synthesis of nicotine. This is in direct contrast to the conclusions of Mothes (1938), who worked with intact tobacco plants; but it does not invalidate his data, for they actually become more susceptible of interpretation when the nicotine content of a leaf is viewed not as a result of synthesis *in situ* but as a result of translocation.

During the growth of the tobacco scions the nicotine which was present in the original scions at the time of preparing the grafts remained in the lower portions of these scions and was not translocated to other parts of the plant. Thus the new growth which

⁶ Similar results have been obtained at the Experimental Institute Leonardo Angeloni for tobacco cultivation at Scafati (Naples) according to a news item reported by Raffaele Sansone in the News Edition of Industrial and Engineering Chemistry for March 20, 1939.

appeared was entirely nicotine free. The absence of translocation outward once the nicotine has made its appearance in the leaf is in complete accord with the results of Mothes. There was no indication that nicotine was either used up by the metabolic processes of the leaf or lost from the leaf by volatilization. The former possibility has also been eliminated by Mothes. The second possibility has, however, been supported by Chazé (1931) in connection with the fate of nicotine in the intact tobacco plant. The present data do not seem to substantiate the view of Chazé.

The distribution of nicotine between stem and leaf in the tomato scions was quite similar to that which occurs in the intact tobacco plant. That is, the stems remained relatively low in nicotine content, while the amount of alkaloid in the leaves increased to a comparatively high value. This point together with the observation that nicotine accumulated unilaterally in those tomato plants which had been grafted laterally to tobacco stocks appears to complete the evidence for the translocatory nature of the origin of the alkaloid in the leaves.

A question arises at this point, however, with regard to whether nicotine was translocated as such or as some precursor or intermediate which might have been converted directly to the alkaloid once it had been deposited in the leaf cells. Definite evidence for one or the other of these possibilities must await the completion of experiments in which the various tissues of the stem are to be separated from one another and analyzed for nicotine. If the first alternative is the correct one, then nicotine should be found in appreciable amounts in either the xylem or the phloem. If the latter alternative is more nearly true, the nicotine of the stem should be found only in the living cells of the cortex or pith or in the cambium.⁷

If the events which have just been described can be taken as indications of the actual behavior of nicotine not only in the reciprocal grafts between tobacco and tomato but also in the intact tobacco plant, many of the apparently contradictory data which are re-

⁷ Since the manuscript was submitted, nicotine has been isolated as the dipicrate in good yield from the xylem of tobacco stalks and also from the sap which bleeds from the xylem of cut stumps of tobacco stalks. The complete data from these experiments will appear elsewhere.

corded in the literature regarding the alkaloidal content of the leaves may be reconciled. It would then appear that nicotine is synthesized in the tobacco root and that it is translocated through the stalk to the leaf where it accumulates. The nicotine content of the tobacco leaf would thus be determined by the net effect of a number of variables some of which would be (1) the size, age, and activity of the root system, (2) the relative size of the shoot, and (3) the total amount of organic carbon and of inorganic nitrogen supplied to the roots by the aerial portion of the plant and the soil, respectively. Whether such relationships actually occur must be established by more direct experimentation. Investigations are now in progress in this laboratory in which the nicotine synthetic capacity of excised tobacco roots in sterile culture is receiving some consideration.

SUMMARY

The distribution of nicotine between stock and scion in reciprocal grafts of tobacco and tomato has been studied.

When tobacco scions were grown upon tomato stocks no appreciable accumulation of nicotine occurred in the tobacco leaves or stems. In fact, the nicotine which was originally present in the scion remained in the lower leaves and stem, and the leaves and stem tissues which subsequently developed were nicotine free.

When tomato scions were grown upon tobacco stocks nicotine was found in small quantities in the tomato stems and fruits, and large quantities of the alkaloid accumulated in the leaves. Nicotine accumulation in the leaves of tomato shoots was sectoral when tobacco stems were decapitated and inserted into the tomato stems unilaterally.

Growth, including both mitosis and cell enlargement, occurred quite normally in the absence of detectable amounts of nicotine when tobacco shoots were grafted upon tomato stocks. It appears, therefore, that nicotine, in appreciable quantities at least, is not essential for the development of the aerial portions of the tobacco plant.

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RELATION OF SEED WEIGHT TO VEGETATIVE GROWTH, DIFFERENTIATION, AND YIELD IN PLANTS¹

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For a period of approximately three hundred years plant propagators have worked with methods for separating seeds into classes, according to weight (Yokoi, 1903). During the past century many investigations have been made on the subject, most of the papers appearing during the last fifty years, prior to 1930. Certain investigators (Schmidt, 1924; Cummings, 1914; Kiesselbach, 1924; Delassus, 1911) have reported results indicating that plants produced from heavier seeds show more vigorous growth, attain greater weight and size, and produce larger yields as determined by number and size of fruits than do plants grown from seeds of lighter weight. On the other hand others have shown by the results of their experiments that the seed weight factor is significant with respect to size of plants only during the early stages of plant growth, and that, if the growing season is long enough, the early superiority might disappear entirely (Golinska, 1929; Kotowski, 1929; Rohmeder, 1939; Vanselow, 1933).

This paper describes studies made to determine the relation of the initial dry weight of seeds to subsequent plant growth. The weights of the seeds were correlated with growth in stem length, leaf areas, dry weight of roots and tops, number of leaves, number of buds, number of flowers, and to fruit and seed yield. Studies were also made on tissue development in stems, leaves, roots, and petioles.

EXPERIMENTAL METHODS AND MATERIALS.—Seeds of each of the following were used in these experiments: Soybeans (*Soja max* Piper, var. Biloxi and Mandarin), cucumber (*Cucumis sativus* L., var. Early White Spine), and tomato (*Lycopersicon esculentum* Mill., var. Bonny Best). The plants were grown in the University of Illinois botany greenhouses.

The seeds of soybeans and cucumber were placed into three groups, designated by the letters X, Y, Z, and the seed coats were removed. In the seeds designated by the letter X, none of the food storage tissue was removed; from those seeds designated by the letter Y, approximately half of one cotyledon was severed; and from those seeds designated by the letter Z, one whole cotyledon was cut off. In other words, the seeds in set Y and in set Z contained approximately three-fourths and one-half respectively of the amount of food reserve contained within the seeds of set X. The seeds were then weighed accurately to one-tenth of a milligram and planted in sterilized well mixed loam soil. The pots containing the seeds were watered daily with sterile tap water until the epicotyls had appeared above the surface of the soil. From this time on these plants were watered daily with unsterilized tap water.

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This work was carried out under the direction of Doctor Harry J. Fuller in the Department of Botany of the University of Illinois.

The method for reducing the amount of reserve food material in seeds, as described above, was not used with the tomato because it is difficult to carry out on small seeds. The tomato seeds were planted in sawdust flats and as soon after germination as the cotyledons appeared above the sawdust, cotyledonary tissue was removed as follows: The seedlings of one group, designated as group X, were left whole; one-half of one cotyledon was removed from each of the seedlings of group Y; each of the seedlings of group Z had a whole cotyledon removed. Twenty-four hours later the plants were carefully taken from the flats, washed in tap water, and after the superficial water had been removed with blotting paper, were weighed accurately to one-tenth of a milligram. The flats were kept in the dark from the time the seeds were planted until the seedlings were removed except for a few minutes during which time the cotyledons were cut. The seeds and seedlings were adjusted to oven dry weight by calculation after percentage weight loss had been determined by drying representative samples in an oven at 100°C., until no further decrease in weight occurred. The tomato seedlings were planted in well mixed loam soil and were watered with unsterilized tap water.

Cultural conditions, temperature, light and moisture were controlled and equalized for all plants as far as possible. All plants were grown in 4½-inch pots, except that tomato and cucumber plants used for yield determinations were grown in eight-inch pots. Each plant was labeled to indicate its group and the weight of the seed from which it grew. Corresponding records were made on data charts.

Dry weights of plants or plant parts were determined by placing these into open weighing bottles and drying them in an oven at a temperature of 100°C., until they showed no further loss in weight. The bottles were then closed and after cooling, the plants or plant parts together with the bottles were accurately weighed to one-tenth of a milligram. Corrections for weights of the bottles were made later.

Leaf areas were determined by making outlines of the leaves on paper having thirty-six squares per square inch. The number of squares was determined by means of a calculator, and the value of these was then converted into square centimeters.

Measurements of cross-sections of roots, stems, petioles, leaves, and their respective tissues were made from permanent mounts on slides by means of calibrated ocular micrometers. Tissues, from which sections were made, were taken at comparable measured positions on each plant so as to avoid errors due to positional differences of the tissues. All sections were made from tissues embedded in paraffin.

Correlation coefficients were determined from the formula:

$$C = \frac{S_{xy}}{\sqrt{(Sx^2)(Sy^2)}}$$

in which C represents the correlation coefficient; S the sum of the respective factor or factors; x the deviations of the seed weights or in the tomato the deviations of the seedling weight from the average; y the deviations of the numbers of flowers, buds, leaves, fruits and seeds, weights of various portions of the plants, growth rates, order of ripening of organs or fruit, and other growth criteria from their respective averages. A coefficient between 0 and plus 1 indicates a positive correlation; a value between 0 and minus 1 indicates a negative correlation. In this paper a negative correlation is indicated by a minus sign; a positive correlation by no sign.

RESULTS AND DISCUSSION.—The life cycle of a plant can be divided into two more or less distinct

phases: The first is the vegetative phase in which the plant undergoes extensive vegetative development; the second phase involves the reproductive processes. It was recently emphasized that the reproductive activities of flowering plants consist of two related but physiologically very different phases: flowering and fruiting (Loehwing, 1938).

Correlation coefficients for numbers and weights of plant organs.—One hundred fifty plants were used for each species or variety in making correlation determinations between seed weight and numbers and dry weights of plant organs. The data recorded were taken at the end of the vegetative phase of plant growth as a few flowers made their appearance. The correlation values indicate that there still

TABLE 1. *Correlation coefficients for numbers and weights of plant organs.*

	Biloxi Soybean	Mandarin Soybean	Cucumber	Tomato
Average dry weight of X seeds.....	0.2466 g.	0.1472 g.	0.0241 g.	0.0070 g.
Average dry weight of Y seeds.....	0.1859	0.1246	0.0180	0.0067
Average dry weight of Z seeds.....	0.1247	0.0965	0.0128	0.0064
Growth period (in days).....	35	30	40	40
Flowers				
Average number on X plants.....	5.244	1.666	1.880	1.227
Average number on Y plants.....	5.659	0.311	0.960	0.590
Average number on Z plants.....	3.466	0.088	0.160	0.363
Correlation coefficients (seed weight with flower number).....	0.2299	0.3108	0.3124	0.3439
Unopened flower buds				
Average number on X plants.....	4.000	4.622	16.260	4.136
Average number on Y plants.....	3.454	4.355	16.660	3.409
Average number on Z plants.....	3.377	3.222	8.060	3.681
Correlation coefficients (seed weight with bud number).....	0.2635	0.3789	0.4434	0.1304
Leaves				
Average number on X plants.....	8.511	5.511	6.040	12.500
Average number on Y plants.....	8.068	4.977	5.460	11.227
Average number on Z plants.....	7.933	4.422	4.240	11.363
Correlation coefficients (seed weight with leaf number).....	0.3139	0.5582	0.3502	0.3374
Height				
Average height of X plants.....	86.301 cm.	47.299 cm.	12.446 cm.	51.231 cm.
Average height of Y plants.....	73.037	37.858	12.141	44.795
Average height of Z plants.....	74.168	29.011	5.974	44.795
Correlation coefficients (seed weight with height).....	0.4657	0.5377	0.4294	0.2423
Dry weight of tops				
Average dry weight of X plants.....	1.8901 g.	0.7286 g.	1.4496 g.	2.5712 g.
Average dry weight of Y plants.....	2.0001	0.5519	0.3284	1.6321
Average dry weight of Z plants.....	1.5499	0.3160	0.1230	1.7994
Correlation coefficients (seed weight with weight of tops).....	0.3337	0.6578	0.5553	0.3803
Dry weight of roots				
Average dry weight of X plants.....	0.1733 g.	0.1240 g.	0.2031 g.	0.2566 g.
Average dry weight of Y plants.....	0.1962	0.0945	0.1135	0.2477
Average dry weight of Z plants.....	0.1422	0.0587	0.0360	0.2160
Correlation coefficients (seed weight with weight of roots).....	0.1282	0.4709	0.4345	0.2063
Dry weight of tops and roots				
Average dry weight of X plants.....	2.0413 g.	0.8821 g.	1.6528 g.	2.8279 g.
Average dry weight of Y plants.....	2.1963	0.6431	0.4419	1.8798
Average dry weight of Z plants.....	1.6899	0.3735	0.1591	2.0155
Correlation coefficients (seed weight with total dry weight of plant).....	0.2327	0.5247	0.5525	0.3828

is a fair correlation between seed weight and number and size of plant organs at the beginning of the flowering phase of the growth cycle (table 1).

The correlation between seed weight and number of flowers may be interpreted as a correlation between seed weight and time of flowering rather than between seed weight and number of flowers produced. This is because at this stage of the growth cycle only a few (the earliest flowers) are open.

A higher correlation value exists between seed weight and dry weight of tops than between seed weight and dry weight of roots. When the plant is taken as a whole, *i.e.*, top and root together, the correlation value usually lies somewhere between that obtained when the top and the root are considered separately.

Leaf areas and cross-sectional dimensions of vegetative organs.—Ninety plants of each species or variety were used. Cross-sections of the leaves were made 5 mm. above the basal attachment of the blade to the petiole. Petiole cross-sections were made 2 cm. above the base of the petiole. The cross-sections of the stem were made 2 cm. above the second node, and root cross-sections were taken 1 cm. below the collar.

A fairly close relationship exists between seed weight and leaf areas and, except in the case of the tomato, between seed weight and cross-sectional dimensions of vegetative organs as shown by the correlation values (table 2).

Growth rate.—During early life, the embryo of a seed is dependent for its growth upon the food stored in the cotyledons or endosperm. This food is made

TABLE 2. *Leaf areas and cross-sectional dimensions of vegetative organs.*

	Biloxi Soybean	Mandarin Soybean	Cucumber	Tomato
Average dry weight of X seeds.....	0.2466 g.	0.1472 g.	0.0241 g.	0.0070 g.
Average dry weight of Y seeds.....	0.1859	0.1246	0.0180	0.0067
Average dry weight of Z seeds.....	0.1247	0.0965	0.0128	0.0064
Growth period (in days).....	35	30	40	40
Leaves				
Blade:				
Average thickness per X plant.....	0.148 mm.	0.120 mm.	0.199 mm.	0.083 mm.
Average thickness per Y plant.....	0.137	0.122	0.185	0.077
Average thickness per Z plant.....	0.116	0.113	0.180	0.076
Correlation coefficients (seed weight with thickness).....	0.6910	0.4145	0.4944	0.4179
Midrib:				
Average thickness per X plant.....	0.832 mm.	0.726 mm.	1.267 mm.	0.776 mm.
Average thickness per Y plant.....	0.808	0.659	1.104	0.616
Average thickness per Z plant.....	0.626	0.604	1.089	0.770
Correlation coefficients (seed weight with thickness).....	0.2706	0.4503	0.7090	0.0166
Average width per X plant.....	0.859 mm.	0.809 mm.	1.400 mm.	0.951 mm.
Average width per Y plant.....	0.835	0.690	1.219	0.877
Average width per Z plant.....	0.793	0.639	1.232	0.946
Correlation coefficients (seed weight with width).....	0.3671	0.5666	0.4921	-0.0158
Petioles				
Average diameter per X plant.....	1.575 mm.	1.493 mm.	2.563 mm.	2.496 mm.
Average diameter per Y plant.....	1.444	1.266	2.253	2.636
Average diameter per Z plant.....	1.267	1.082	2.210	2.576
Correlation coefficients (seed weight with diameter).....	0.6868	0.7137	0.4874	-0.1035
Stems				
Average diameter per X plant.....	2.400 mm.	2.372 mm.	3.878 mm.	6.165 mm.
Average diameter per Y plant.....	2.094	1.889	3.538	5.869
Average diameter per Z plant.....	2.025	1.805	3.475	5.995
Correlation coefficients (seed weight with diameter).....	0.5244	0.4732	0.3134	0.1256
Roots				
Average diameter per X plant.....	3.991 mm.	3.148 mm.	4.858 mm.	5.290 mm.
Average diameter per Y plant.....	3.511	2.547	3.973	4.879
Average diameter per Z plant.....	2.964	2.486	3.874	5.154
Correlation coefficients (seed weight with diameter).....	0.6530	0.4957	0.4809	0.2673
Leaf area				
Average in square centimeters per X plant.....	639.044	167.379	142.822	722.239
Average in square centimeters per Y plant.....	600.437	120.478	101.487	480.779
Average in square centimeters per Z plant.....	460.536	64.401	52.507	519.723
Correlation coefficients (seed weight with leaf area).....	0.4904	0.5456	0.4815	0.3750

TABLE 3. *Weekly stem growth-rate correlation coefficients; average weekly increases in stem elongation (in centimeters).*

	Biloxi Soybean	Mandarin Soybean	Cucumber	Tomato
Average dry weight of X seeds.....	0.2479 g.	0.1659 g.	0.0235 g.	0.0070 g.
Average dry weight of Y seeds.....	0.1863	0.1178	0.0165	0.0067
Average dry weight of Z seeds.....	0.1250	0.0783	0.0112	0.0063
First week				
Average increase per X plant.....	2.50	2.50	2.55	2.85
Average increase per Y plant.....	2.50	2.51	2.50	2.75
Average increase per Z plant.....	2.15	1.75	1.75	2.25
Correlation coefficients.....	0.434	0.280	0.735	0.356
Second week				
Average increase per X plant.....	8.00	5.15	3.00	3.55
Average increase per Y plant.....	6.65	5.35	1.67	2.50
Average increase per Z plant.....	5.58	2.80	0.95	2.50
Correlation coefficients.....	0.662	0.470	0.622	0.388
Third week				
Average increase per X plant.....	5.83	6.55	2.90	4.22
Average increase per Y plant.....	5.15	4.88	2.80	3.60
Average increase per Z plant.....	4.90	4.27	1.72	3.75
Correlation coefficients.....	0.494	0.642	0.523	0.217
Fourth week				
Average increase per X plant.....	8.42	6.37	10.07	6.93
Average increase per Y plant.....	6.90	5.20	7.22	6.10
Average increase per Z plant.....	6.57	3.30	5.12	5.95
Correlation coefficients.....	0.576	0.700	0.633	0.334
Fifth week				
Average increase per X plant.....	13.70	22.00	27.50	9.32
Average increase per Y plant.....	11.00	20.57	22.97	9.40
Average increase per Z plant.....	10.97	15.42	14.67	8.65
Correlation coefficients.....	0.477	0.473	0.539	0.101
Sixth week				
Average increase per X plant.....	18.57	13.15	43.85	10.10
Average increase per Y plant.....	18.97	10.92	41.42	8.90
Average increase per Z plant.....	16.75	11.40	40.75	9.70
Correlation coefficients.....	0.244	0.177	0.105	0.060
Seventh week				
Average increase per X plant.....	14.90	9.15	39.50	14.27
Average increase per Y plant.....	16.92	7.45	39.07	14.57
Average increase per Z plant.....	15.05	13.57	40.37	15.27
Correlation coefficients.....	-0.166	-0.209	-0.060	-0.106
Eighth week				
Average increase per X plant.....	7.02	1.65	38.75	13.50
Average increase per Y plant.....	9.22	0.72	36.45	13.85
Average increase per Z plant.....	9.22	3.97	37.92	14.02
Correlation coefficients.....	-0.383	-0.219	0.101	-0.037
Ninth week				
Average increase per X plant.....	2.65	1.50	42.12	11.02
Average increase per Y plant.....	4.07	0.52	42.00	9.55
Average increase per Z plant.....	4.22	2.22	43.37	10.77
Correlation coefficients.....	-0.300	-0.175	-0.073	-0.010
Tenth week				
Average increase per X plant.....	1.00	43.50	9.60
Average increase per Y plant.....	1.70	37.75	9.55
Average increase per Z plant.....	1.55	48.25	10.97
Correlation coefficients.....	-0.145	-0.106	-0.097

available for the embryo by digestion at an increasing rate as the seed germinates and the plant begins to grow. After the leaves unfold and begin to increase in size, photosynthetic activity supplements the storage food supply of the plant. As the plant increases in size, the growth rate also increases up to the initiation of flower primordia or the actual appearance of flowers. At this point the growth rate slows down considerably, partly because more and more of the nutritive supplies formerly used for vegetative growth are used for reproductive functions and partly because of alterations in the condition of growth-promoting and growth-inhibiting substances (McCollum, 1934).

Growth rate correlation coefficients were determined for the relationship between seed weight and weekly increases in stem elongation. Measurements were taken at semi-weekly intervals, but computations were made on a weekly basis. One hundred fifty plants were used for each species or variety (table 3).

During the first week of the growth period there is very little difference in the growth rate between the plants from the different seed weight groups. It has been stated that the metabolic translocations of reserve food material from the cotyledons to the growing plant is more efficient in the seedling containing the smaller amount of stored food (Gould,

TABLE 4. *Seed weight and tissue differentiation.*

TABLE 4. Seed weight and tissue dry weight							
	Biloxi Soybean		Mandarin Soybean		Tomato		
Average dry weight per X seed.....	0.2466 g.		0.1472 g.		0.0070 g.		
Average dry weight per Y seed.....	0.1859		0.1246		0.0067		
Average dry weight per Z seed.....	0.1247		0.0965		0.0064		
Growth period (in days).....	35		30		40		
Principal bundle in midrib of leaf							
	mm.	mm.	mm.	mm.	mm.	mm.	
Average size per X plant.....	0.227×0.490		0.175×0.486		0.187×0.550		
Average size per Y plant.....	0.199×0.467		0.167×0.357		0.167×0.522		
Average size per Z plant.....	0.172×0.433		0.171×0.334		0.162×0.520		
Bundles in petioles							
Average size per X plant.....	0.138×0.186		0.128×0.168		0.282×0.975		
Average size per Y plant.....	0.127×0.167		0.104×0.135		0.267×0.915		
Average size per Z plant.....	0.117×0.150		0.091×0.129		0.260×0.865		
Stem							
	Area	%	Area	%	Area	%	
	sq. mm.	area	sq. mm.	area	sq. mm.	area	
Pith:							
Average area per X plant.....	1.01	22	1.17	26	14.05	47	
Average area per Y plant.....	0.98	28	0.91	32	10.84	40	
Average area per Z plant.....	0.85	27	0.88	35	12.31	43	
Xylem:							
Average area per X plant.....	2.25	50	1.54	35	4.85	16	
Average area per Y plant.....	1.56	45	0.94	33	3.92	15	
Average area per Z plant.....	1.41	44	0.79	31	4.19	15	
Tissues outside xylem:							
Average area per X plant.....	1.27	28	1.72	39	10.95	37	
Average area per Y plant.....	0.91	27	0.98	35	12.28	45	
Average area per Z plant.....	0.94	29	0.87	34	11.71	42	
Root							
Pith:							
Average area per X plant.....	0.53	4	0.61	8	0.73	3	
Average area per Y plant.....	0.48	5	0.41	8	0.42	2	
Average area per Z plant.....	0.36	5	0.32	7	0.79	4	
Xylem:							
Average area per X plant.....	3.02	25	1.88	24	6.92	32	
Average area per Y plant.....	2.24	23	1.00	20	5.80	31	
Average area per Z plant.....	1.68	24	1.01	21	5.74	27	
Tissues outside xylem:							
Average area per X plant.....	9.01	71	5.25	68	14.33	65	
Average area per Y plant.....	6.89	72	3.65	72	12.47	67	
Average area per Z plant.....	4.84	71	3.51	72	14.33	69	

TABLE 4. *Concluded.*

	X=0.0241 g. Seed weight Y=0.0180 Z=0.0128	Number of vessel-like elements	Diameter of vessel-like elements mm.	Size of vascular bundles mm. mm.
Cucumber				
Midrib of leaf:				
Average per X plant.....		30.80	0.030	0.377 × 0.419
Average per Y plant.....		25.87	0.026	0.316 × 0.369
Average per Z plant.....		26.02	0.023	0.311 × 0.357
Petiole:				
Average per X plant.....		37.40	0.038	0.206 × 0.380
Average per Y plant.....		45.62	0.035	0.184 × 0.331
Average per Z plant.....		40.43	0.035	0.180 × 0.318
Stem:				
Average per X plant.....		73.61	0.068	0.325 × 0.516
Average per Y plant.....		59.20	0.062	0.260 × 0.454
Average per Z plant.....		60.67	0.056	0.256 × 0.446
Root:				
Average per X plant.....		64.19	0.061	0.685 × 1.334
Average per Y plant.....		54.09	0.056	0.609 × 1.090
Average per Z plant.....		56.13	0.056	0.565 × 1.047

et al., 1934). In general the highest correlation values between seed weight and rate of stem elongation are found for the second, third, and fourth week.

Except for the tomato, the values are still fairly high for the fifth week. The sixth and seventh weeks show marked decreases in all values. The negative

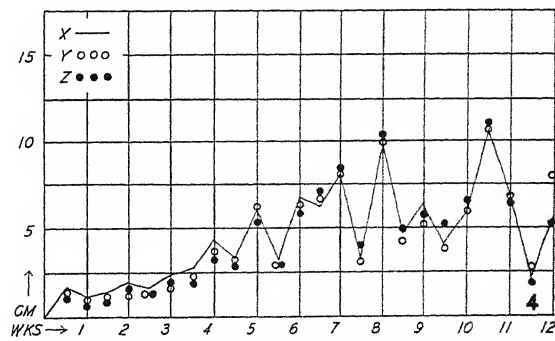
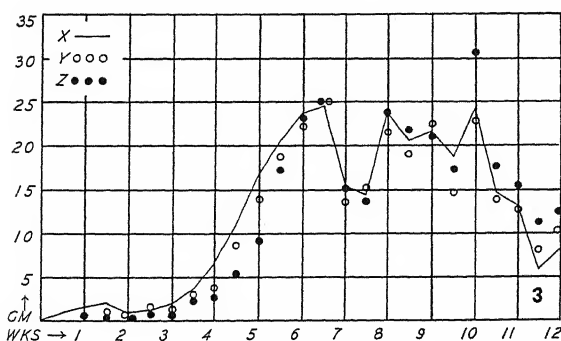
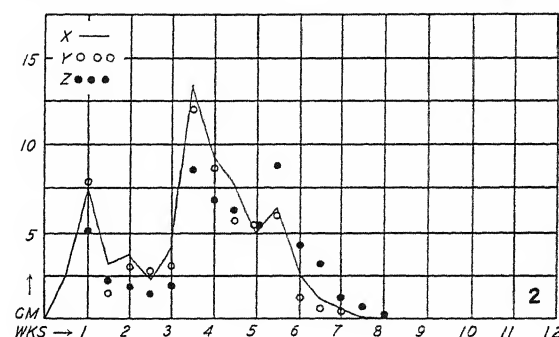
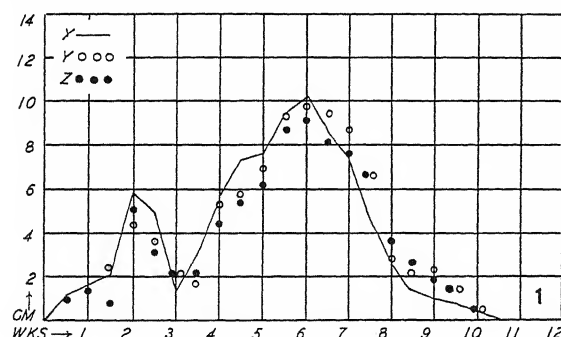


Fig. 1-4. Increment growth curves.—Fig. 1. Growth curves for Biloxi soybeans.—Fig. 2. Growth curves for Mandarin soybeans.—Fig. 3. Growth curves for cucumber.—Fig. 4. Growth curves for tomato. X represents growth curve for control plants grown from whole seeds; Y represents growth curve for plants grown from seeds with one-half of one cotyledon removed; Z represents growth curve for plants grown from seeds with a whole cotyledon removed.

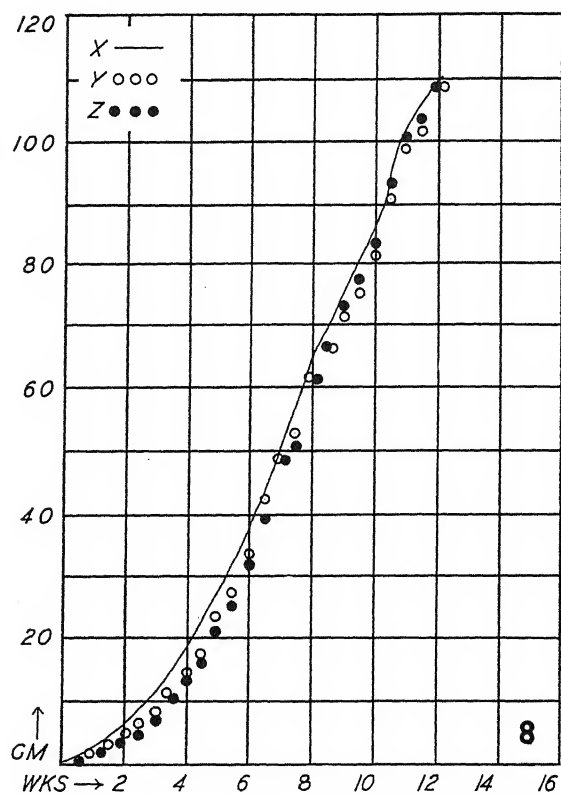
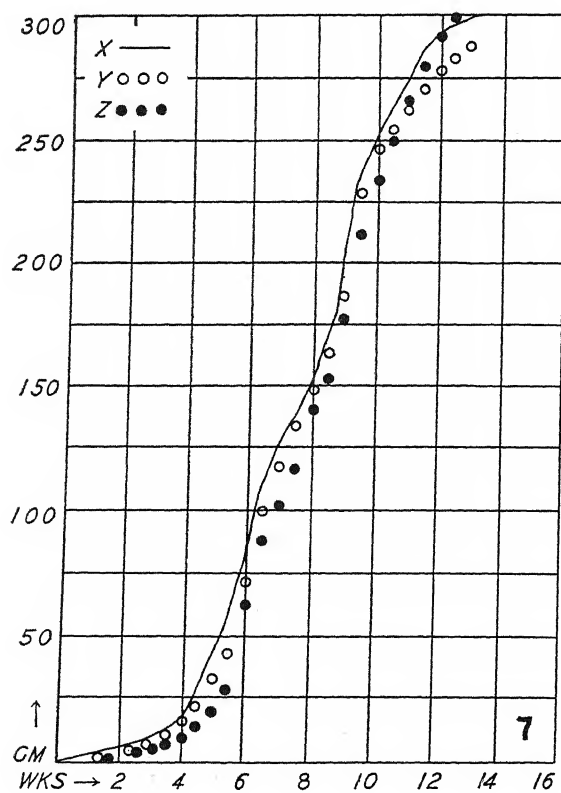
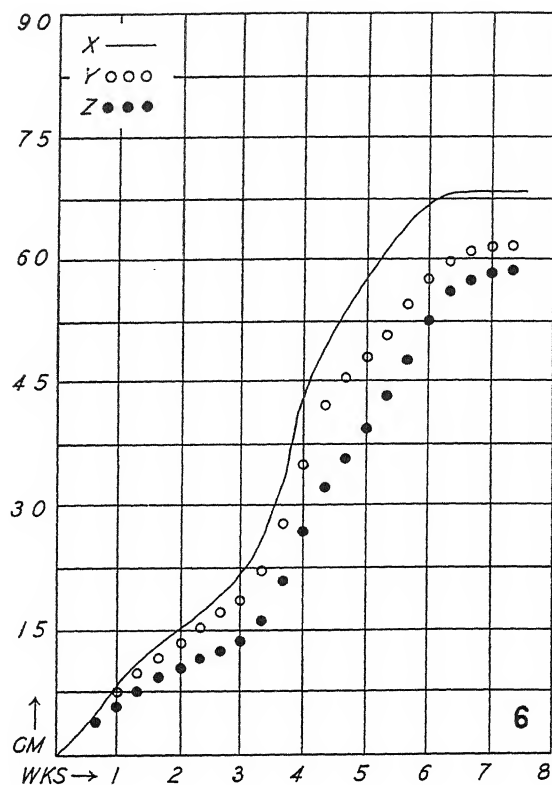
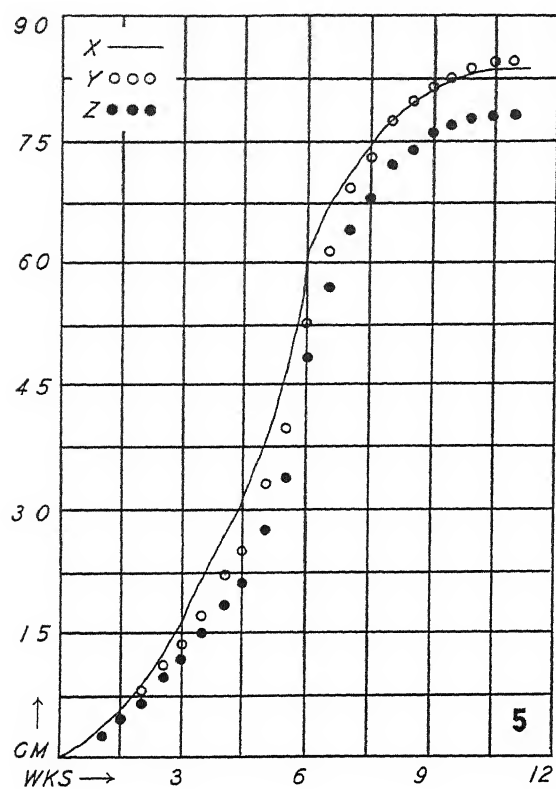


TABLE 5. *Correlation coefficients: seed weight with yield.*

	Biloxi Soybean	Mandarin Soybean	Cucumber	Tomato
Average dry weight of X seeds.....	0.2497 g.	0.1659 g.	0.0235 g.	0.0070 g.
Average dry weight of Y seeds.....	0.1863	0.1178	0.0165	0.0067
Average dry weight of Z seeds.....	0.1250	0.0783	0.0112	0.0063
Fruits				
Average number per X plant.....	3.54	8.75	1.25	8.14
Average number per Y plant.....	3.70	7.70	1.10	7.42
Average number per Z plant.....	3.37	8.91	0.75	7.57
Correlation coefficients	0.030	0.006	0.226	0.079
Dry weights of seeds and fruits				
	Seeds		Fruits	
Total average weight per X plant.....	1.1874 g.	1.4623 g.	12.342 g.	27.496 g.
Total average weight per Y plant.....	1.1575	1.4257	12.811	26.515
Total average weight per Z plant.....	0.8047	1.7465	10.020	27.109
Correlation coefficients	0.315	-0.109	0.113	0.157
Individual average weight per X plant.....	0.2045 g.	0.1010 g.	10.325 g.	3.913 g.
Individual average weight per Y plant.....	0.2174	0.1067	12.035	4.078
Individual average weight per Z plant.....	0.1474	0.1144	12.731	3.783
Correlation coefficients	0.436	-0.295	-0.061	0.064
Number of seeds per pod				
Average number per X plant.....	1.395	1.705
Average number per Y plant.....	1.483	1.750
Average number per Z plant.....	1.383	1.679
Correlation coefficients	-0.004	-0.026
Number of seeds per plant				
Average number per X plant.....	5.250	14.666
Average number per Y plant.....	5.416	13.458
Average number per Z plant.....	4.875	15.217
Correlation coefficients	-0.028	-0.075

values, beginning with the seventh week, progressively increase for a period of time, depending on the time required for the plants to reach maturity. Usually near the end of the growing period the values again swing toward the positive side (fig. 1-4; table 3). Golinska (1929) has pointed out that the length of the growth period and the speed of development of a plant are hereditary and cannot be influenced much by seed weight. Heredity thus seems to be responsible for the shift in correlation values during the sixth or seventh week of the growth period.

The values obtained by measurements made of total stem length at semi-weekly intervals were plot-

ted against time, giving sigmoid growth curves (fig. 5-8). The progress of these plants is more easily seen by plotting the average increment at semi-weekly intervals against time, thus giving graphic representations of the grand period of growth (fig. 1-4).

There seems to be little or no relationship between seed weight and height of plants at maturity. Plants from seeds of lighter weight may in some instances surpass in height the plants grown from heavier seeds (fig. 5-8). The early superiority of plants from heavier seeds over that of plants from lighter seeds gradually disappears, and, if the growing season is long enough, the early differences may disappear en-

TABLE 6. *Seed weight and order of tomato ripening.*

	First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Remainder
Average number per X plant.....	0.409	1.000	0.363	2.045	0.818	0.454	2.681
Average number per Y plant.....	0.454	0.954	0.362	1.771	1.367	0.545	1.590
Average number per Z plant.....	0.636	0.454	0.681	2.045	0.772	0.272	2.363
Correlation coefficients	-0.130	0.254	-0.175	-0.005	0.033	0.112	0.042

Fig. 5-8. Sigmoid growth curves.—Fig. 5. Growth curves for Biloxi soybeans.—Fig. 6. Growth curves for Mandarin soybeans.—Fig. 7. Growth curves for cucumber.—Fig. 8. Growth curves for tomato. X represents growth curve for control plants grown from whole seeds; Y represents growth curve for plants grown from seeds with one-half of one cotyledon removed; Z represents growth curve for plants grown from seeds with a whole cotyledon removed.

tirely (Kotowski, 1929; Rohmeder, 1939; Vanselow, 1933).

At the termination of the experiments the growth period had not yet ended for the cucumber and the tomato (fig. 3, 4, 7, and 8). The reasons for not carrying these experiments to completion were: (1) These plants have a very long growth period under greenhouse conditions. (2) During the latter part of the period, the growth becomes rather irregular due to fruiting conditions and hereditary factors. (3) The work was carried out far enough to show the fundamentals involved, namely, that the plants from the lighter seeds have a slower initial growth rate, but that their growth rate later usually surpasses that of the plants from the heavier seeds.

The percentage of cotyledonary tissue removed from seeds appears to have a smaller effect on the early growth rate of a plant when the seeds are large than when they are smaller. There is also less effect when the true leaves come early in the development of the plant. In Biloxi soybeans which have relatively heavy seeds and a relatively early and proportionate development of photosynthetic surface, the effect on the growth rate by the removal of a certain percentage of storage tissue is relatively small. Mandarin soybeans have seeds of lighter weights and a smaller development of early photosynthetic surface; here the reduction of seed weight has a greater effect on the growth rate. Cucumbers have seeds of relatively light weight and a relatively late development of true leaves; here the effect produced by the removal of a certain percentage of storage tissue on the early growth is still greater than in Mandarin soybeans. The relation between the percentage of storage tissue removed from seeds and early plant growth appears to be dependent, therefore, on the initial weight of the seed and on the time and speed of development of photosynthetic surface.

Seed weight and tissue differentiation.—Thirty plants were used for each species or variety. Sections were made as noted above.

The results indicate that the reduction in size of various tissues and the decrease in number and size of vessel-like elements, due to the removal of different portions of reserve food tissues, is not proportional to the amount of such tissue removed, at least not beyond a certain point. In general there is a greater degree of difference in reduction between plants of group X and plants of group Y than between plants of group Y and plants of group Z (table 4).

There is a closer relationship between seed weight and actual cross-sectional areas of tissues than between seed weight and the relative development of the various tissues in a section (table 4). In other words, when reduction in tissue development occurs, the tissues are all reduced in approximately the same ratio; thus the percentage values remain more or less constant. In the percentage distribution of tissues, seed weight generally has the greatest effect on xylem development.

Except for vascular bundle number, which according to these experiments, in the species used, is nearly a constant and is not affected by seed weight, the results are in harmony with those reported in the literature (Delassus, 1913). Delassus found that in the plants with which he worked (beans, vetches, lupins) the vascular bundle number decreased with a decrease in reserve food in seeds from which the plants grew. This fact was not substantiated by the author's experiments.

Seed weight and yield.—There is considerable controversy as to whether larger and heavier seeds will produce plants with greater yielding capacities. Certain reports in the literature indicate that larger and heavier seeds produce plants which in general produce yields superior in dry weight, numbers of fruit, or in quality to those produced from lighter seeds (Fikry, 1936; Cummings, 1914; Staffeld, 1926). On the contrary other papers present results which show little or no relationship between seed weight and yield (Kotowski, 1929; Montgomery, 1908; Rotunno, 1924).

In the experiments reported here, ninety plants of each species or variety were used for yield determinations. The soybean fruits were harvested after the plants had dried naturally and the cucumber fruits at the end of ninety days. The tomato fruits were harvested as they ripened, beginning about the one hundred tenth day and ending about the one hundred sixtieth day. The tomatoes remaining on the vines after the sixth week of ripening were picked green. Dry weights and numbers of fruits and seeds were correlated with the dry weights of the seeds from which the plants were grown (tables 5 and 6).

All the correlation values are very low with two exceptions, both in Biloxi soybeans, these exceptions being in the correlation between: (1) seed weight and total average weight of seeds produced per plant, and (2) seed weight and individual average seed weight per plant. These exceptions do not seem to be significant, however, because: (1) they are but two in number; and (2) there seems to be little difference in yield between the plants originating from the heaviest seeds and those coming from the medium weight class. The difference seems to be in the yield produced by the plants from the lightest seed weight group. The correlation values between seed weight and order of tomato ripening are low, thus showing no relationship.

Seed weight in relation to mortality rate and disease resistance.—It has been reported in the literature that plants grown from heavier seeds seem to be less susceptible to rusts than plants grown from seeds of lighter weight (Norton, 1913; Delassus, 1911). Vanselow (1933) pointed out that plants from lighter weight seeds show a higher mortality rate. In the experiments reported in this paper, no study was made of the relation of disease resistance to seed weight, but it was observed that the mortality rate was higher among the plants from the lighter seeds as compared to the plants from the heavier seeds. In soybeans the percentage of mortality was

2 per cent in the plants from the heavy seeds (Biloxi 0.2466 g.; Mandarin 0.1472 g.), 3.5 per cent in plants from the medium weight seeds (Biloxi 0.1859 g.; Mandarin 0.1246 g.), and 11 per cent in the plants from the light seeds (Biloxi 0.1247 g.; Mandarin 0.0965 g.). Cucumber plants from heavy and medium weight seeds (0.0241 g. and 0.0180 g.) showed 3 per cent mortality as compared to 8 per cent mortality in plants from light seeds (0.0128 g.). In tomato there was a mortality of about 3 per cent with no noticeable relation to seed weight. The mortality rate, in all cases, reached its peak during the second or third week.

SUMMARY

It has been shown that there is a positive correlation between the initial seed weight and number, size, dry weight and cross-sectional dimensions of the various organs of the plants used in these experiments at the beginning of the flowering phase of the growth cycle. The values of such correlation vary among the different species and varieties of plants used in these experiments.

The importance of the seed weight factor is greatest during the early stages of plant growth. In tomatoes, cucumbers, and soybeans, the plants grown from the lighter seeds have a slower initial growth rate which persists till about the end of the sixth week; thus there is a positive correlation for this period of growth. Beginning with the seventh week and generally continuing to the end of the growth period, the growth rate for the plants grown from the lighter seeds exceeds that for the plants grown from the heavier seeds; thus the correlation values for this period are generally negative. Under favorable

greenhouse conditions the early superiority of plants grown from heavier seeds over those grown from lighter seeds is generally lost by the time the plants reach maturity.

The relation between the percentage of storage tissue removed from seeds and early plant growth seems to be dependent on the natural weight of seeds and on the time and speed of the development of photosynthetic surface.

The plant tissues (pith, xylem, and tissues outside the xylem) are all reduced in cross-sectional area in approximately the same ratio.

There appears to be a relation between seed weight and number and size of vessel-like elements. There is also a reduction between seed weight and vascular bundle size (length and width as seen in cross-section), but not between seed weight and vascular bundle number. Vascular bundle number varies with the species and thus seems to be a hereditary characteristic.

When the plants used in these experiments are grown under favorable greenhouse conditions, there is but little relation between seed weight and yield (numbers, sizes, and dry weights of fruits and seeds). No relationship was found between seed weight and order of fruit ripening in the tomato.

A higher mortality rate was found among seedlings grown from seeds of lighter weight than among seedlings grown from heavier seeds. This difference in mortality rate is probably due to differences in plant vigor and disease susceptibility.

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NORTHFIELD, MINNESOTA

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STUDIES IN THE GENUS *SCIRPUS* L. IV. THE SECTION BOLBOSCHOENUS PALLA¹

Alan A. Beetle

THE LEAFY, perennial bulrushes comprising the section *Bolboschoenus*, subgenus *Euscirpus* (Beetle, 1940) of the genus *Scirpus* (Cyperaceae), are common in temperate regions of the Northern Hemisphere between latitudes 30° and 50°. Outside of this area they are rare except for a concentration in Australia. The six species and two varieties recognized here form a closely related group of extremely variable species. The universal tendency to confuse all entities with the Linnaean *S. maritimus* has obscured an accurate understanding of their ranges and affinities.

Inasmuch as the haploid number 55 has been reported for *Scirpus maritimus*, *S. paludosus*, *S. fluviatilis*, and *S. robustus*, it is presumed that this is not an aneuploid series as is the case in certain other sections of *Scirpus* (e.g., Section *Lacustres*). Reports of other numbers, all smaller, for these species are probably incorrect and due to the extreme technical difficulties involved.

The longevity and easy viability of *Scirpus* achenes lead inevitably to a wide distribution of the species. Thus the occurrence of odd distributional patterns or of occasional extra-limital localities should not be a cause for undue concern. *Scirpus robustus* occurs naturally all along the Atlantic coast of North America but suddenly reappears near the two largest west coast seaports at Long Beach and around San Francisco Bay. The Eurasian *S. tuberosus* occurs near both New York and New Orleans. Surely these localities represent introductions over shipping lanes.

The species listed here are of great importance in wild life conservation, the large and abundant achenes constituting an important bird food. The species may also be of some significance as soil binders on river estuaries or along the coast but are not otherwise of major economic importance.

In the preparation of this paper material in the herbaria of the following institutions has been consulted: Gray Herbarium (G), Harvard University; Rocky Mountain Herbarium (R.M.), University of Wyoming; University of California (U.C.); The Academy of Natural Sciences of Philadelphia; United States National Herbarium (U.S.); New York Botanical Garden (N.Y.); California Academy of Sciences; Dudley Herbarium, Stanford University; Field Museum (F.M.), Chicago; Missouri Botanical Garden (M.O.), St. Louis; and the author's personal collection. In the interest of brevity the source of sheets will not be further specified, except in the case of types.

TAXONOMIC TREATMENT.—Section *Bolboschoenus* Palla in Koch, Syn. Deutsch. Fl. 3: 2531. 1904. Perennials; joints of the rootstocks often enlarged into hard woody tubers; stems leafy; involucre of folia-

ceous bracts; spikelets terminal, sessile or umbellate; stamens 3; style 2- or 3-fid; achene trigonous, compressed, or lenticular, smooth under low magnification, gray or brown, apiculate; bristles usually present. (Type species: *Scirpus maritimus* L.)

Key to the Species

- Achenes lenticular; style 2-fid.
 - Spikelets 3 to many, often pedicellate. 1. *S. paludosus*
 - Spikelets 1-3, all sessile. 2. *S. strobilinus*
- Achenes compressed- to sharply trigonous; style 2- or 3-fid.
 - Leaf normally not over 6 mm. broad; secondary roots not conspicuous.
 - Style normally 2-fid; spikelets ovate-acute. 3. *S. maritimus*
 - Spikelets several.
 - Spikelets solitary. 3. *S. maritimus* forma *monostachyus*
 - Style normally 3-fid.
 - Spikelets oval. 4. *S. maritimus* var. *fernaldi*
 - Spikelets broadly cylindrical. 3. *S. maritimus* forma *macrostachyus*
 - Spikelets narrowly cylindrical, acute. 5. *S. tuberosus*
- Leaf commonly 8-10 mm. broad; secondary rootlets conspicuous.
 - Spikelets few, mostly sessile, or single on short rays. 6. *S. robustus*
 - Spikelets many, one to several on long rays.
 - Achenes dark brown, 3 mm. long. 7. *S. robustus* var. *novae-angliae*
 - Achenes dull gray-brown, 4 mm. long. 8. *S. fluviatilis*

1. *SCIRPUS PALUDOSUS* A. Nels.
 - Scirpus campestris* Britt. in Britt. & Brown, Ill. Fl. 1: 267. 1866; not Roth (1795).
 - Scirpus paludosus* A. Nels. Bull. Torrey Club 26: 5. 1899.
 - Scirpus robustus* var. *campestris* Fern. Rhodora 2: 241. 1900.
 - Scirpus robustus* var. *paludosus* Fern. op. cit. 1900.
 - Scirpus robustus* var. *compactus* Davy ex Jepson, Fl. W. Mid. Calif. 88. 1901.
 - Scirpus pacificus* Britt. ex Parish, Bull. South. Calif. Acad. Sci. 4: 132. 1905.
 - Scirpus interior* Britt., Man. Fl. N. U. S. ed. 2, 178, 1905.
 - Scirpus brittonianus* Piper, Cont. U. S. Nat. Herb. 11: 157. 1906.
 - Scirpus campestris* var. *paludosus* Fern., Rhodora 8: 162. 1906.
 - Scirpus maritimus* var. *paludosus* Kuenth. ex Fedde, Report. Spec. Nov. 23: 200. 1926.

Culms 0.5-1.5 m. tall, erect from smooth, brown, horizontal rhizomes, thickened at the nodes, frequently forming tubers; roots slightly spongy, the secondary roots inconspicuous; culms sheathed half their height, sharply trigonous, smooth; leaf sheaths

¹ Received for publication August 18, 1941.

green, the hyaline orifice abruptly truncate, the blades 3–5 cm. long, commonly 8 mm. broad, the margins and midrib scabrous at tip only; bracts of the inflorescence 2–5, leaf-like, unequal, the outer up to 2 cm. long, the others progressively shorter, the margins and midrib scabrous; inflorescence of 3 to many spikelets, 1–2.5 cm. long, 0.5 cm. broad, ovate to cylindric-acute, sessile or umbellate; primary rays up to 5 cm. long, smooth; scales ca. 6 mm. long, the midrib prolonged 2 mm. beyond the bifurcate apex, light brown, hairy; bristles 2–6, about half length of achene, fragile, minutely barbed; stamens 3, the style 2-fid, 6 mm. long; achenes very variable in size, 3–4 mm. long, 2 mm. broad, light to dark brown, ovate, lenticular, apiculate. April–August.

Type from Granger, Wyoming, *Aven Nelson* 3874 (R.M.).

Range: Fresh to strongly saline water; on the Atlantic Coast from New Brunswick to New Jersey, occasionally inland in Quebec and New York; from Saskatchewan south to Texas and west to the Pacific Coast, Hawaiian Islands, Argentina.

CANADA: Quebec: Magdalen Is., *Fernald et al.* 6981 & 6983 & 4459; Colchester Co., Truro, *Fernald & Wiegand* 4248; Vercheres Co., Varennes, *Victorin* 28,652; Rimouski Co., Rimouski, *Svenson & Fassett* 1004.

Nova Scotia: Hants Co., St. Croix River, *Prince & Atwood* 1024; Sable Island, *St. John* 1160; Shelburne Co., Jordan Falls, *Fernald & Long* 23,397.

New Brunswick: Gloucester Co., Bathurst, *Blake* 5457.

Saskatchewan: Saskatoon, *Fraser* 10; St. Front, *Breitung* 11; Little Manitou Lake, *Macoun & Herriot* 73,101.

Alberta: Cragmyle Dist., *Brinkman* 314.

British Columbia: Vancouver Is., *Macoun* 7546 & 78784 & 78785.

MAINE: York Co., Kittery, *Fernald & Long* 12843; Lincoln Co., *P. Wilson* 35; Hancock Co., *Brenner* 8250; Knox Co., *Steyermark* 716 & 4051.

MASSACHUSETTS: Barnstable Co., Yarmouth, *Fernald & Long* 18059; Plymouth Co., Plymouth, *Blake* 3777; Nantucket Co., *Pennell* 11135; Dukes Co., Elizabeth Is., *Fogg* 2288 & 487.

RHODE ISLAND: Washington Co., Westerly, *Weatherby & Collins* in 1919.

CONNECTICUT: Fairfield Co., Fairfield, *Eames* in 1898; New London Co., Old Lyme, *Woodward* in 1917; New Haven Co., Madison, *Sargent* in 1934.

NEW YORK: Onondaga Co., Syracuse, *Wiegand* 5929, Kirkwood in 1903, *Drushel* 8745; Seneca Co., Seneca Falls, *Wiegand & Eames* 9363; Long Island, *Bicknell* 1164, 1169, 1183, 1184, *Ferguson* 3225, 3275, 4055, 4930.

NEW JERSEY: Great Island, *Vail* in 1890, Weehawken, *Van Sickle* in 1894; Middlesex Co., South Amboy, *Mackenzie* 1372.

EAST INDIES: *Ekman* 1325.

MINNESOTA: Big Stone Co., *Moyle* 2317; Lyon Co., *Hotchkiss & Jones* 328, 3992, 3982.

NORTH DAKOTA: Richland Co., Hankinson, *Metcalf* 159; Cass Co., Fargo, *Stevens* in 1910; Benson Co., *Lunell*.

SOUTH DAKOTA: Spink Co., Redfield, *Ricksecker* 185; Stanley Co., Interior, *Over* 6250; Hardin Co., n. of Crook, *Fisher* 79.

NEBRASKA: Kearney Co., Minden, *Hapeman* in 1930; Cass Co., n.e. of Louisville, *J. L. Morrison* 1177; Box Butte Co., Alliance, *Webber* 8.

KANSAS: Barton Co., Ellinwood, *Benke* 5122, Great Bend, *Benke* 5123; Reno Co., *Hitchcock* in 1897; Stafford Co., *Carleton* 278.

OKLAHOMA: Harper Co., *Stevens* 288; Woods Co., near Alva, *Stevens* 1607; Alfalfa Co., near Cherokee, *G. W. Stevens* 623.

MISSOURI: Saline Co., s.w. of Ridge Prairie, *Steyermark* 21587.

TEXAS: Bailey Co., Coyote Lake, *Ferris & Duncan* 3462; El Paso Co., between Fabens & Ysleta, *Ferris & Duncan* 2363; Reeves Co., San Solomon Springs, *Cory* 9657; Reeves Co., Balmohrea, *Hinckley* 1076.

MONTANA: Phillips Co., Malta, *Blankinship* in 1901; Westby, *Larsen* 107; Cascade Co., Great Falls, *Sperry & Martin* 713.

WYOMING: Albany Co., Laramie Plains, *Nelson* 8189; Sweetwater Co., Green River, *Everman* in 1893; Sheridan Co., Sheridan, *Nelson* 9593; Carbon Co., Saratoga, *Nelson* 9661.

COLORADO: Kiowa Co., s. of Eads, *Beetle* 2054; Fremont Co., Canon City, *Brandegge* 447; Weld Co., Windsor, *Osterhout* 7978; Huerfano Co., Walsenburg, *Shear* 4759.

NEW MEXICO: Sierra Co., Derry, *Crowell* 10; Dona Ana Co., Mesquite, *Fosberg* S3926; Eddy Co., Carlsbad, *Fisher* 38200.

IDAHO: Canyon Co., Emmett, *Macbride* 886; American Falls Reservoir, *Sperry & Martin* in 1929.

UTAH: Box Elder Co., Kelton, *Wetmore* 450; Garfield Beach, *Rydberg & Carlton* 6908; Carbon Co., Price, *Stanton* 979; Washington Co., Zion Park, *Boyle* 2317.

ARIZONA: Maricopa Co., Tempe, *Peebles* 9317; Pinal Co., Florence, *Toumey* 503; Navajo Co., Winslow, *Griffiths* 5029.

NEVADA: Nye Co., Beattie, *Heller* 10424; Churchill Co., Fallon, *Tidestrom* 10728; Elko Co., Ruby Valley, *Heller* 9469.

WASHINGTON: Douglas Co., jct. Crab & Wilson Creeks, *Sandberg & Leiberg* 333; King Co., Seattle, *Piper* in 1891; Jefferson Co., Townsend, *Otis* 2338.

OREGON: Harney Co., e. of Burns, *Thompson* 13283; Lake Co., s. of Adel, *Applegate* 7553; Douglas Co., mouth of Umpqua River, *Howell* in 1887.

CALIFORNIA: Alameda Co., vic. of Berkeley, *Beetle* 2630; Mendocino Co., near Mendocino, *H. E. Brown* 846; Los Angeles Co., Long Beach, *Parish* in 1891 (type of *Scirpus pacificus*); Solano Co., Cordelia, *Jepson* 12, 934.

MEXICO: Baja Calif., Seven Wells on the Salton River, *Mearns* 2870; valley of the Lower Rio

Grande, Buckley in 1878-1883; Tecate River, Schoenfeldt 3725.

ARGENTINA: Gob. Chubut, s. of Trelew, Eyer-dam et al. 23589; Rio Negro Gob., vic. of General Roca, Fischer 176; Capital Federal, Puerto Nuevo, Barros 2177.

HAWAIIAN ISLANDS: Island Oahu, Heller 2208, Mani. Degener 9027; Honolulu, Hitchcock 13808.

The strangely disrupted range of this species may explain in part some of the difficulty which has been experienced in attempting to treat it satisfactorily. The confusion lies principally in attempting to distinguish between *Scirpus maritimus* and *S. paludosus*. Fernald indicated that all the referable North American material must be segregated from the European, whereas Mackenzie, recognizing that true *Scirpus maritimus* occurred in North America, wished to relegate to that species all the collections of these two species from the northeastern coast. Both apparently overemphasized the principle of geographic isolation as a clue to speciation in closely related groups. Actually both *S. maritimus* and *S. paludosus* occur in this area. Although not easily distinguished they may be separated by a number of characters, including the shallowly bifurcate apex of the scale, the usually narrow leaves, and the compressed-trigonus achene of *S. maritimus* as opposed to the deeply bifurcate apex of the scale, the usually broader leaves, and the wholly lenticular achene of *S. paludosus*.

This species exhibits only moderate variation. In some areas the majority of the specimens appear to have light as opposed to dark brown scales (e.g., the Rocky Mountain region, although the type of *Scirpus paludosus* is not remarkable in this respect), in others the spikelets are frequently rayed instead of sessile (particularly along the Pacific coast), and again the swollen tubers on the roots may be mostly absent (New England). None of these represent varieties of the strength accepted by the author.

2. *SCIRPUS STROBILINUS* Roxb.

Scirpus strobilinus Roxb. Hort. Beng. 6. 1814, nomen nudum; Fl. Ind. ed. Carey & Wall. 1: 222. 1820.

Scirpus affinis Roth, Nov. Pl. Sp. 30. 1821.

Scirpus maritimus var. *affinis* Clarke in Hook. Fl. Brit. India 6: 659. 1894.

Scirpus balna Ham. ex Clarke op. cit. 1894, nomen in synonym.

Bolboschoenus affinis Drob. in Trav. Mus. Bot. Acad. Petersb. 16: 139. 1916.

Culms up to 5 dm. tall, erect from slender, smooth, horizontal rhizomes; roots often spongy, the secondary rootlets inconspicuous, the culms thickened at their base, sheathed to one-half their height, sharply trigonous, sometimes obscurely scabrous at the summit, leafy, the sheaths green, the orifice truncate, blades to 2 dm. long, 5 mm. or less broad, the margins scabrous near the tip; bracts of the inflorescence 1-3, unequal, the outer up to 1 dm. long, scabrous-mar-

gined; spikelets one to 5, usually not more than 3, sessile, 2 mm. long, 1 mm. broad, ovate, light brown, the scales 7 mm. long, 4 mm. broad, light brown, pubescent, mucronate from a bifurcate apex; bristles 3 to 6, fragile, shorter than the achene; style 2-fid; achene lenticular, dark brown, ovate, apiculate, 3 mm. long, 2 mm. broad. October.

Type from India, "ad ripas Gangia," presumably in the Herbarium of the Kew Botanical Gardens.

Range: India and Turkestan.

INDIA: Punjab, Drummond 24,934, Stewart 12,501 & 13,411; East Bengal, Herb. Griffith.

Roxburgh's original description of *Scirpus strobilinus*, although short, seems amply to justify the application of the name to this species. The complete diagnosis is as follows: "Sc. radice stolonifera, culmo trigono basi folioso, foliis triangularibus canaliculatis, spica laterali solitaria, involucri monophyllo, squamis cordatis cuspidatis, stigmatibus bifido, setis hypogynis senis glabris. Hab. ad ripas Gangia." Furthermore, this is not the first time that the name has been connected with this entity. C. B. Clarke (see synonymy listed above) at the time he proposed the combination *Scirpus maritimus* var. *affinis* listed *S. strobilinus* in the synonymy.

3. *SCIRPUS MARITIMUS* L.

Scirpus maritimus L. Sp. Pl. 1: 51. 1753.

Scirpus capensis Burm. f. Fl. Cap. Prod. 3. 1768.

Scirpus mucronatus Pollich. Hist. Pl. Palat. 1: 44. 1776-77.

Scirpus cyperoides Lam. Fl. Fr. 3: 653. 1778.

Scirpus glaucus Lam. Tabl. Encyc. et Meth. 1: 142. 1791.

Scirpus macrostachyus Lam. op. cit. 1791.

Scirpus compactus Hoffm. Deutschl. Fl. ed. II. 1: 1: 25. 1804.

Scirpus maritimus (riparius) Pers. Syn. 1: 68. 1805.

Scirpus maritimus var. *macrostachyus* Pers. op. cit. 1805.

Scirpus maritimus var. *cymosus* Reich. Fl. Germ. 79. 1830-32.

Scirpus vulgaris (Mazziari) in Ionios Anthol. 2: 460. 1834.

Scirpus maritimus var. *glaucus* Wight, Contrib. bot. Ind. 111. 1834.

Scirpus maritimus var. *cylindricus* Wight, op. cit. 1834.

Scirpus decumans Willd. ex Kunth, Enum. Pl. 2: 168. 1837.

Scirpus swampanus Bose ex Kunth, op. cit. 1837.

Scirpus lucidus Less ex Kunth, op. cit. 1837.

Scirpus salinus Schmidt, ex Steud. Nom. ed. II. 2: 541. 1840-41.

Scirpus maritimus var. *compactus* Kroch, in Reich. Ic. Fl. Germ. et Helv. 8: 43. 1846.

Scirpus maritimus var. *umbellatus* Reich. op. cit. 1846.

Scirpus maritimus var. *monostachyus* Sonder, Fl. Hamb. 27. 1851.

Reigera maritima Opiz, Sezman 83. 1852.

Scirpus megastachyus Steud. Syn. Pl. Cyp. 87. 1855.

Scirpus squarrosulus Steud. *op. cit.* 88. 1855.

Isolepis grandispica Steud. *op. cit.* 318. 1855.

Scirpus ehrenbergii Boeckl. Linnaea 36: 716. 1869-70.

Scirpus laciniatus Nees & Ehr. ex Boeck. *op. cit.* 1869-70.

Scirpus hyalinolepis Steud. ex Jardin, Bull. Soc. Linn. Normand. Ser. II. 9: 278. 1875.

Scirpus nobilis Ridl. Trans. Linn. Soc. ser. 2. 2: 159. 1884.

Scirpus koshewnikowii Litw. In the list of Wild Plants of the Tambov Gov. 142. 1888.

Bolboschoenus maritimus Palla, in Koch. Syn. III. 2532. 1904.

Bolboschoenus compactus Drob., Trav. Mus. Bot. Acad. Petersb. 11: 92. 1913.

Bolboschoenus macrostachys Grossh. Flora Caucasus 1: 145. 1928.

Scirpus ewersanii Fisch. ex Litw. Acta Hort. Petrop. 40: 271. 1929.

Culms up to 8 dm. tall, often 1 cm., in depauperate forms, slender, erect from smooth, horizontal rhizomes, the roots slightly spongy, only at the nodes; the secondary rootlets inconspicuous; culms thickened at the base, sheathed at least half their height, sharply trigonous, at most only very obscurely scabrous, near the summit; very leafy, the sheaths green, the orifice truncate, hyaline, the blades mostly 5 dm. long, seldom over 5 mm. wide, the margins and midrib scabrous at tips; bracts of the inflorescence 1-5, leaflike, unequal, the outer up to 2 cm. long, the others progressively shorter, margins and midrib scabrous; inflorescence of one to many spikelets 1-3.5 cm. long, 5 mm. broad, oval to ovate acute, sessile or umbellate; primary rays up to 4.5 cm. long, smooth, the scales ca. 7 mm. long, the midrib prolonged 2 mm. beyond the bifurcate apex, brown, minutely hairy; bristles 2, nearly equaling the achene, retrorsely barbed; stamens 3; style 2-fid, or rarely 3-fid, 6 mm. long, achenes 3.5 mm. long, 2 mm. broad, dark brown, shiny, punctulate, compressed-trigonous. August-September.

Type in the Linnaean Herbarium.

Range: throughout Europe, occasional in Asia; Atlantic coast of North America from Nova Scotia to New York.

EURASIA: sheets seen from Russia, Roumania, Denmark, Sweden, Finland, Germany, France, England, Spain, Switzerland; rare in Australia, Japan, China, Siberia where probably introduced.

CANADA: New Brunswick, Northumberland Co., Blake 5707; Charlotte Co., Whale Cove, Weatherby & Weatherby 5609.

PRINCE EDWARD ISLANDS: Queens Co., Fernald et al. 6980.

NOVA SCOTIA: Truro, Fassett 2194; Shelburne Co., Jordan Falls, Fernald & Long 23398; Colchester Co., Fernald & Wiegand 4249.

MAINE: Washington Co., Moose Island, Fernald 1413; Knox Co., South Thomaston, Bissell et al. 8943; York, Bicknell 1156.

NEW HAMPSHIRE: Sea Brook, Eaton 507; Portsmouth, Davis in 1894; Hampton Falls, Eaton in 1898.

MASSACHUSETTS: Essex Co., Herb. Oakes; Scituate, Rich in 1901; Egypt, Williams in 1901.

RHODE ISLAND: Olney.

CONNECTICUT: Milford, Eames 39.

NEW YORK: Long Beach, Bicknell.

NEW JERSEY: Camden, Martindale.

The widely accepted interpretation of typical *Scirpus maritimus* is adopted here because it appears to agree with a photograph of the material in the Linnaean Herbarium. Two striking variations (among a host of lesser ones) occur throughout the species population. Because these are wholly without geographical identity and may be interconnected at many localities with the mean of the species and with each other, they are given only formal rank. The variation with a single sessile spikelet on a plant of reduced stature may be called *Scirpus maritimus* forma *monostachyus* (Sonder.). The other, of large size throughout and with a broadly cylindric-acute, usually rayed spikelets may be called *S. maritimus* forma *macrostachyus* (Lamb.).

4. *SCIRPUS maritimus* L. var. *fernaldi* (Bicknell) Beetle comb. nov.

Scirpus fernaldi Bicknell, Torrey 1: 96. 1901.

Scirpus campestris var. *fernaldi* Bartlett, Rhodora 8: 163. 1906.

Like the species but spikelets ovate, 1-2 cm. long, 1-1.5 cm. broad, sessile or 1-3 on smooth rays 3-5 cm. long; the achenes sharply trigonous.

Range: northeastern coast of North America from New Brunswick and Nova Scotia south to Maine and Massachusetts.

Type locality, Mt. Desert, Maine, August 20, 1898, Bicknell (N.Y.).

CANADA: New Brunswick, Gloucester Co., Miscous Is., Blake 5584; Nova Scotia, Cape Breton Is., Baddeck, Macoun 20,771; Colchester Co., Fassett 2193; Prince Edward Is., Summerside, Fernald et al. 6985, Mt. Stewart, Fernald et al. 6984; Quebec, Magdalen Island, Grindstone Is., Fernald 6986 & 6983.

MAINE: Washington Co., Whiting, Knowlton in 1916; Bristol, Chamberlin 695.

MASSACHUSETTS: Banks of Mystic River, Medford, W. Boott in 1859.

The trigonous achene and 3-fid style are especially significant in indicating that this entity is closely related to the European *S. maritimus* rather than to the American *S. paludosus*.

5. *SCIRPUS TUBEROSUS* Desf.

Scirpus corymbosus Forskal. Fl. Aeg.-Arab. 14. 1775; not L., 1756.

Scirpus tuberosus Desf. Fl. Atl. 1: 50. 1798-1800.

Scirpus aegyptiacus Poir. Encyc. Method. 6: 770. 1804.

Scirpus maritimus var. *tuberosus* R. & S. Syst. Veg. 2: 138. 1817.

Scirpus tridentatus Roxb. Fl. Ind. ed. Carey & Wall. 1: 228. 1820.

Scirpus cephalotes Heyne ex Roth. Nov. Pl. Sp. 30. 1821.

Culms up to 5 dm. tall, slender, erect from swollen nodes on slender black rhizomes; roots spongy, the secondary rootlets inconspicuous; culms sheathed to at least one-half their length, sharply trigonous, very obscurely scabrous at summit; very leafy, the sheaths green, the orifice conspicuously raised, hyaline, the blades mostly less than 5 mm. broad, margins and midrib scabrous at the tip; bracts of the inflorescence unequal the outer to 1 dm. long, the margins scabrous; inflorescence compound umbellate, of many spikelets, 1-2 cm. long, smooth, scales 7 mm. long, pubescent, light to dark brown, the apex notched, the midrib mucronate; bristles 6, fragile, unequal, shorter than the achene, retrorsely barbed; stamens 3, anthers 3 mm. long; style 3-fid, or rarely in the same spikelets 2-fid; achenes 2.5 mm. long, 2 mm. broad, trigonous, dark brown, slightly apiculate. June.

Type presumably somewhere in Europe. A collection from Algeria, Prov. de Oran, Coll. G.-L. Durando in 1852, specimen in the Gautier Herbarium of H. Knoche, San Jose, California, is here pointed out to be representative, as clearly as that is possible for one specimen.

Range: from Spain and Algeria along the coast of the Mediterranean Sea to the Caucasus; India.

Distribution: Eurasian specimens seen from France, Algeria, Syria, Central Asia (Trtysch Valley), Caucasus, and India (Punjab).

NEW YORK: Long Island, Queens, *Ferguson* 2396.

ALABAMA: *C. Mohr* in 1868.

The North American localities almost certainly represent random introductions.

Early in the study of this section of *Scirpus* it became evident that two specific entities were included in *S. maritimus* as it has been interpreted by European authors. True *S. maritimus* is most abundant on the northern coasts of France and Germany. A second species occurs around the Mediterranean Sea but never spreads far into northern Europe. The name *S. tuberosus* has been chosen for the latter for two reasons: (1) it is the first name which fits the plant applied to it in a region where it grows to the exclusion of *S. maritimus*, and (2) although the species is based principally upon a fallacious conception of the root system, the original description specifically mentions the shape of the spikelets as being different from the spikelets of *S. maritimus*.

6. *SCIRPUS ROBUSTUS* Pursh.

Scirpus maritimus var. *macrostachyus* Michx. Fl. Bor. Amer. 1: 32. 1803.

Scirpus macrostachyus Muhl. ex Spreng. Syst. Veg. 1: 211, Gram. 45. 1817, not Lam., 1791.

Scirpus robustus Pursh. Fl. Amer. Sept. 1: 56. 1814.

Scirpus maritimus var. *cylindricus* Torr. N. Y. Acad. Sci. 3: 323-325. 1836.

Scirpus decumans Willd. ex Kunth, Enum. Pl. 2: 168. 1837.

Scirpus strobiliferus Steud. Syn. Pl. Glum. Pars. 2, 317. 1855.

Scirpus maritimus var. *robustus* Kükenthal, ex Fedde, Repert. Spec. Nov. 23: 200. 1926.

Culms about 1 m. tall, stout, erect from swollen nodes on a smooth, black, horizontal rhizome; roots fibrous only at the nodes, the secondary rootlets numerous; culms trigonous, smooth, leafy nearly to the summit; leaf sheaths green, the orifice hyaline-margined and raised, the blades up to 6 dm. long, commonly 1 cm. broad, smooth below but frequently scabrous on the margins and midrib at the summit; bracts of the inflorescence 2-4, unequal, the outer up to 3 dm. long, the others progressively shorter; the margins and midrib scabrous, the spikelets 1-3 cm. long, 1 cm. broad, 3 to many, sessile or umbellate, single on short rays, ovate cylindric; primary rays up to 3 cm. long; smooth, scales 6 mm. long, 4 mm. broad, brown, minutely hairy, the strongly recurved midrib prolonged 2 mm. beyond the apex which sometimes appears bifurcate through splitting; bristles variable; style 3-fid; achene 3 mm. long, 2 mm. broad, lenticular or ovate, apiculate, dark brown, shiny. June-October.

Type not located; Pursh listed no specimens or localities in his original description. A collection from Florida, Duval County, Jacksonville, June 6, 1896, *A. H. Curtis* (U.C.) is here pointed out as representative of the species. This specimen has been chosen because (1) it is complete; (2) it is from a part of the range of *Scirpus robustus* not included in the ranges of other species in the section; (3) the collection is widely distributed (R.M., N.Y., Mo., G., F.M., U.S.).

Range: Atlantic coast of North America from Nova Scotia to Mexico, Bahamas, Brazil, Paraguay, Argentina; California.

CANADA: Nova Scotia, Victoria Co., Baddeck Bay, *Fernald & Long* 20, 215; New Brunswick, Gloucester Co., Bathurst, *S. F. Blake* 5457.

MAINE: Hancock Co., Franklin, *Friesner* 4649.

NEW HAMPSHIRE: Rockingham Co., Sea Brook, *Eaton* 507.

MASSACHUSETTS: Barnstable Co., Harwich, *Fernald & Long* 16378, Orleans, *Murdock* 705; Nantucket Co., *Pennell* 11,136; Essex Co., Newbury, 1898, *Williams*; Middlesex Co., Cambridge, 1912, *Kennedy*.

RHODE ISLAND: *Short*; 1891, *Congdon*; Newport Co., Block Island, *Fernald & Long* 8931.

CONNECTICUT: New London Co., Norwich, 1884, *Setchell*; Lyme, 1929, *Jansson*; New Haven Co., Milford, 1895 & 1898, *Eames*, *Blewitt* 3455.

NEW YORK: Suffolk Co., Southampton, *St. John* 2612; Queens Co., College Point, *Pennell* 7558;

Nassau Co., Jones Beach, *Gleason & Smith 144*; Long Island, *Ferguson 728, 2502, 7691 & 3274*.

NEW JERSEY: Cape May Co., *Mackenzie 6343*, Cold Spring, *Pennell 1819*; Cumberland Co., Sea Breeze, *J. W. & M. T. Adams 2027*; Ocean Co., Waretown, *Mackenzie 4807*.

DELAWARE: New Castle Co., Pea Patch Is., *Larsen 604*; Kent Co., Fraland Beach, *Larsen 613*, Bowers Beach, *Pennell 12327*.

MARYLAND: Worcester Co., n. of Ocean City, *Fogg 11,393*; Dorchester Co., Cambridge, *House 233*; Calvert Co., Chesapeake Beach, *Chase 2523*.

VIRGINIA: Westmoreland Co., Colonial Beach, *Dowell 1587*; Warwick Co., Newport News, *Leonard & Killip 78*; James City Co., n. of Williamsburg, *Grimes 3868*; Princess Anne Co., Long Is., *Fernald & Long 10,973*.

NORTH CAROLINA: Cartaret Co., Bogue, *Godfrey 5795*; Hyde Co., Englehard, *Godfrey 4340*; Currituck Club, *McAtee 1197*.

SOUTH CAROLINA: Georgetown Co., Georgetown, *Godfrey & Tryon 339, 342 & 353*, *Alexander 116*, Santa Club, *Alexander 61*; Charleston Co., s. of McClellanville, *Correll 5325*.

GEORGIA: Camden Co., White Oak River, *Harper 1157*; Assabaw Island, *Sperry 569*.

FLORIDA: Duval Co., Jacksonville, *Curtis 4787 & 5680*; Walton Co., *A. H. Howell 593*; Brevard Co., *Fredholm 5602*; Collier Co., Everglades, *Deam 58, 654*; Calhoun Co., St. Vincent Is., *McAtee 1849 & 1783*.

ALABAMA: Mobile Co., *Mohr 1880*.

LOUISIANA: Terrebonne Co., below Houma, *Belanger* in 1913; Monteer, *Tracy 1583*; Plaqueminas Co., 1878, *Langlois*; Rainy Refuge, *Sperry 366*.

MISSISSIPPI: Harrison Co., Biloxi, *Tracy 3589 & 5362*, Ocean Springs, *Tracy 104*, 1895, *Skehan*; Mississippi Delta and adj. isls., *Lloyd & Tracy 398*.

TEXAS: Harris Co., Seabrook, 1917, *Fisher*; Galveston Co., San Leon, *Fisher 1540*; Mueces Co., near Corpus Christi, *Wolff 2341*; Jefferson Co., Port Arthur, McFadden Ranch, *Martin & Warren* in 1930.

CALIFORNIA: Solano Co., Suisun Marshes, *Jepson 21,220*; Sonoma Co., Napa, *Jepson 21,219*; Marin Co., near San Rafael, *Davy 4040*, *Beetle 1754*; Orange Co., Newport Lagoon, *Booth 1105*, *Ewan 7728*.

BAHAMAS: Inagua, *Nash & Taylor 1245*.

MEXICO: vic. of Tampico, *Palmer 160*, *Roviroso 736*.

BRAZIL: *Gardner 1205*.

PARAGUAY: Pilcomayo River, *Morong 927*.

ARGENTINA: Gob. Formosa, Riacho Pilaga, *Wetmore 758*.

7. *SCIRPUS robustus* Pursh var. *novae-angliae* (Britt.) Beetle comb. nov.

Scirpus novae-angliae Britt. in Britt. & Brown, Ill. Fl. 3: 509. 1898.

Scirpus campestris var. *novae-angliae* Fern. Rhodora 8: 163. 1906.

Like the species but the inflorescence more ample, the spikelets 6 mm. broad, up to 4 cm. long, cylindric-acute, many, sessile or on smooth rays, the primary up to 8 cm., the secondary up to 4 cm.; achene trigonous, dull brown.

Range: rare in coastal estuaries from Maine to Virginia.

Type locality, Connecticut, Fairfield, a fresh water marsh bordering creek, tide-water setting back to this point, July 19, 1896, *E. H. Eames* (N.Y.).

MAINE: Sagadahoc Co., Woolwich, *Fernald & Long 12,847*; Falmouth, *Fellows 5925*.

MASSACHUSETTS: Barnstable Co., Dennis, *Fernald & Long 18,061*, Barnstable, *Fernald & Long 18,062*.

RHODE ISLAND: Block Island, *Fernald & Long 8934*.

CONNECTICUT: Fairfield Co., Fairfield, 1896, *Eames*; Stratford, 1897, *Eames*; Lyme, *Harger 6601*.

NEW YORK: Westchester Co., between Glenwood & Hastings, *Bicknell 1174*; Long Island, Wading River, 1877, *Miller*.

NEW JERSEY: Salem Co., s.w. of Harrisonville, *Long 45,263*.

DELAWARE: Wilmington, 1866, *Commons*, Kent Co., Milford, 1908, *Long & Van Pelt*.

MARYLAND: Caroline Co., *Tatnall 2966*.

VIRGINIA: James City Co., Black River, *Fernald & Long 10,972*; Surry Co., e. of Scotland, *Fernald & Long 8593*.

The broad leaves, numerous secondary roots, coastal distribution, 3-fid style, and achenes all indicate the close relationship of this entity to *Scirpus robustus* rather than to *S. paludosus*.

8. *SCIRPUS FLUVIATILIS* (Torr.) Gray.

Scirpus maritimus var. *fluvialis* Torr. Ann. Lyc. N. Y. 3: 325. 1836.

Scirpus fluvialis Gray. Man. ed. 1: 527. 1848.

Culms 1-1.5 m. tall, stout, erect from swollen nodes on the horizontal rhizome; roots not spongy, the secondary rootlets numerous; culms trigonous, sheathed to one-half their height, smooth; leaf sheaths green, becoming dark brown, the orifice truncate, chartaceous; blades up to 6 dm. long; commonly 1 cm. wide, scabrous on the margins but the midrib smooth; bracts of the inflorescence 3 to 5, leaf-like, unequal, the outer up to 2.5 dm. long, the others progressively shorter, the margins and midrib scabrous; inflorescence umbellate, the spikelets 1-2.5 cm. long, to 1 cm. broad, ovate-acute, smooth; scales ca. 8 mm. long, the midrib prolonged 2 mm. from the tapered apex, brown, minutely hairy; bristles 6, variable, often equaling the achene, retrorsely barbed; stamens 3; style 3-fid, 1.5 cm. long; achene 4 mm. long, 2.25 mm. broad, trigonous, dull, gray-brown, punctulate, prominently apiculate, June-July.

Type locality "common in the western parts of N. Y., Gray" (N.Y.).

Range, throughout temperate North America; eastern Asia south to New Zealand, and New South Wales.

CANADA: New Brunswick, Sunbury Co., Burton, *Fassett* 2198; Kings Co., Westfield, *Fernald* 1412, *Fassett* 2200; Quebec, La Prairie Co., St. Lawrence River, *Svenson & Fassett* 1009; Montreal, *Victorin & Germain* 49,307; Ontario, Ottawa, *J. Macoun* 7542, *Rolland* 6202; Kingston, 1881, *Fowler*; 1893, *McMorine*; Saskatchewan, Pike Lake, *Fraser* 6.

MAINE: Sagadahoc Co., Bowdoinham, *Fassett* 156 & 30, *Fernald & Long* 12,842.

VERMONT: Otter Creek, 1879, *Brainerd*; Ferrisburg, 1885, *Morong*; Charlotte, 1911, *Dutton*; Highgate, 1873, *Eggleston*.

MASSACHUSETTS: Nantucket Island, *Bicknell* 1175; Essex Co., Amesbury, *Eaton* 173; Lawrence, 1877, *Robinson*; Wayland, 1910, *G. G. Kennedy*; Barnstable Co., East Brewster, *Fernald & Long* 18,060.

CONNECTICUT: Hartford Co., East Windsor, *Bissell* 268; Fairfield Co., Stratford, 1898, *Eames*; Middlesex Co., East Haddam, *Weatherby* 4292.

NEW YORK: Tompkins Co., Ithaca Flats, 1904, *Woglum*; Albany Co., *House* 6572; Seneca Co., Seneca Falls, *Eames & Ruckes* 3582; Schenectady Co., Scotia, *Muenschner & Clausen* 4416.

PENNSYLVANIA: Erie Co., Presque Isle, 1928, *Witz*; Guttenberg in 1880; Delaware Co., 1866, *A. H. Smith*.

NEW JERSEY: Burlington Co., Fieldsboro, *Long* 18,844; Swedesboro, *Lippincott* 128; Beverly, *Mackenzie* 6166.

MARYLAND: Cecil County, Elkton, *Pennell* 13,293; Benner in 1932; Caroline Co., Dover Bridge, *Tatnall* 2959; Anne Arundel Co., Hall's Bridge, *Shreve* 1549.

DELAWARE: Wilmington, 1867, *Canby*; New Castle Co., Augustine Beach, *Pennell* 7809.

DISTRICT OF COLUMBIA: *Steele* in 1897, *Ward* in 1878, *Chickering*.

OHIO: Lake Co., Painesville, *Beardslee*; Erie Co., Marietta, 1895, *Moseley*; Put-in-Bay, 1898, *Peters*.

INDIANA: Wells Co., Harrison, 1907, *Deam*; Noble Co., near Ligonier, *Deam* 14320.

MICHIGAN: St. Clair Co., St. Clair, 1893, *Wheeler*.

WISCONSIN: Brown Co., Green Bay, *Schuette*; Dane Co., Roxbury, *Fassett* 2792; Polk Co., Balsom Lake, 1897, *Burlllehaus*; Door Co., Moss Bay, 1883, *Schuette*.

ILLINOIS: Start Co., n.e. of Wady Petra, *Chase* 51; St. Clair Co., 1879, *Eggert*; Lake Co., Beach, *Gates* 2785; Cook Co., Chicago, *Gates* 386.

MINNESOTA: Clearwater Co., Itasca Park, *Grant* 3364; Nicollet Co., Nicollet, 1892, *Ballard*; Dakota Co., Lakeville Lake, 1893, *Sheldon*.

IOWA: Winnebago Co., Forest City, 1896, *Shimek*; Emmet Co., Armstrong, 1897 & 1898, *Cratty*; Clay Co., Elk Lake, *Hayden* 56.

MISSOURI: Jackson Co., Sheffield, *Mackenzie* 851; Adair Co., n. of Noringer, *Palmer & Steyermark* 41,142; Bates Co., w. of Papinsville, *Steyermark* 21,284; Lewis Co., n. of Canton, *Steyermark* 26,487.

NORTH DAKOTA: Leeds, *Lunell* in 1899, 1902 & 1907; Cass Co., Fargo, 1933, *Stevens*; Grand Forks, *Brannon* 119; Wade, *Bell* 310.

SOUTH DAKOTA: Kingsbury Co., Iroquois, 1894, *Thorner*; Brookings Co., Brookings, 1894, *Thorner*; Brown Co., Aberdeen, *Griffiths* 852; Clay Co., Vermillion, *Over* 5046.

NEBRASKA: Kearney Co., Platte River, *Rydberg* 4680; Grant Co., n.e. of Whitman, *Rydberg* 1612; Cedar Co., St. James, *Clements* 2634.

KANSAS: Topeka, in 1898; Saline Co., *Carleton* in 1892.

MONTANA: Box Elder Co., 1900, *Blankinship*.

NEW MEXICO: Rio Arriba Co., vic. of Lake Burford, *Wetmore* 514.

IDAHO: Kootenai Co., Lake Coeur d'Alene, *Henderson* 4609.

WASHINGTON: Klickitat Co., Bingen, *Suksdorf* 6419; King Co., Lake Sammanish, *Otis* 1683.

OREGON: Klamath Co., Klamath Falls, *Applegate* 756; n.e. of Keno, *Peck* 9427, Klamath Lake, *Walpole* 2313.

CALIFORNIA: Sutter Co., *Copeland* 3263; Lassen Co., *Davy* 3313, 3285 & 3291; San Joaquin Co., n.e. of Stockton, *Abrams* 6742; San Mateo Co., Crystal Springs Lake, *Hayward* in 1927.

Specimens have also been seen from New South Wales, New Zealand, Japan, China and Siberia. These specimens agree essentially with the North American material in the achene but differ in the bifurcate apex of the scale.

SUMMARY

For the Section *Bolboschoenus* Palla of the Cyperaceous genus *Scirpus* six species and two varieties are recognized. A description, the complete synonymy and known geographic range are given for each species. *Scirpus strobilinus* Roxb. (1820) is upheld as an earlier name for the usually accepted *S. affinis* Roth (1821). *Scirpus tuberosus* Desf. which is usually included with *S. maritimus*, is recognized as distinct. Evidence for the newly suggested relationships implied by *Scirpus maritimus* var. *fernaldi* and *S. robustus* var. *novae-angliae* is given.

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WATER LOSS FROM EXCISED LEAVES ¹

John Shafer, Jr.

IN STUDIES of physiological differences between various inbred and hybrid strains of corn (*Zea Mays*, L.), it is desirable to know if there are differences between the strains with respect to the water loss from the leaves, especially when transpiration is independent of possible differences in supply from the roots. This has application in studies of photosynthesis under conditions of high light intensity and rapid water loss, for consequent stomatal closure may interfere with assimilation. To determine if such differences exist, rates of water loss from excised leaves were determined by successive weighings.

The technic employed was to grow two inbreds and their hybrid side by side in pots in the greenhouse. No attempt was made to control environmental conditions. Immediately prior to an experiment, one healthy looking seedling plant of each strain was selected, and the potted plants were brought to the laboratory. The second, third or fourth leaves developed were used. Leaves were chosen which were fully developed but not old. Generally, the next higher leaf was almost fully expanded when the selected leaf was used. Usually only one leaf was used from each plant, but occasionally tests were made with a second leaf taken about one week after the first had been used. For any one experiment the leaves were all of the same position on the stem, *e.g.*, third from the base. The leaves were cut off in the laboratory, one at a time, and weighed. Subsequently, the leaves were weighed in turn again and again. The humidity of the laboratory was neither measured nor controlled; the light in the laboratory was diffuse. Since a weighing took about two minutes, each leaf was weighed every six or seven minutes. Between weighings the leaves were placed on galvanized iron wire mesh supported several inches above the desk top. The first weighing of each leaf was usually two minutes after cutting, and all leaves had been cut and

weighed once within ten or fifteen minutes after the plants had been removed from the greenhouse. After a few weighings, one weighing was omitted while leaf areas were determined with a planimeter. The leaves were weighed repeatedly for about 11½ hours, after which they were dried in a vacuum oven at 85°C. for several days and their dry weights determined. From the data were calculated total water loss per square inch, fresh weight per square inch, dry weight per square inch, percentage of dry matter in the fresh leaf, percentage of water in the fresh leaf, and percentage of water in the leaf at the start of "cuticular transpiration." Cuticular transpiration is the term used, in agreement with Stålfelt's terminology (1932, 1935), for the low and apparently constant transpiration rate attained eventually by the excised leaves.

The various strains differ from each other with respect to some of the statistics calculated. Since the differences seem independent of the inbred or hybrid nature of the plants, only a summary table of the results is presented here (table 1).

Each individual figure in table 1 is the average of ten or more separate values. All of the figures concerning any one set of strains (a set consisting of two inbreds and a hybrid) are averages of the same number of individual values. However, the different sets of strains were not employed the same number of times. Moreover, the different sets were grown and weighed at different times of the year. Consequently, caution must be exercised in comparing the data of any one set with those of the others.

Figure 1 is a graph of data from a typical experiment. Since most of the experiments were begun just afternoon on bright days, it is probable, in conformity with the work of Stålfelt (1932) and Pfeiderer (1933), that the initial rapid decrease in the rate of transpiration is due to a rapid closure of stomates as the water content of the leaf decreases. If this is

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TABLE 1. Data concerning water and dry matter relationships of corn leaves.^a

Pedigree of plants	Total H ₂ O loss per sq. in.	Fresh wt. per sq. in.	Dry wt. per sq. in.	% H ₂ O fresh	% H ₂ O at start of cuticular transpiration	
	mg.	mg.	mg.			
#1 inbred	19.0	99.0	10.5	89.2	86.2	Vigorous hybrid
#2 inbred	7.4	87.9	9.5	89.0	88.6	
1×2 hybrid	10.5	91.5	9.9	88.8	87.8	
193 inbred	15.0	123.4	11.4	90.8	89.7	Vigorous hybrid
194 inbred	9.5	102.7	9.6	90.7	90.0	
193×194 hybrid	10.2	106.3	9.7	90.9	90.0	
190 inbred	16.1	83.8	11.3	86.3	83.3	Weak hybrid
191 inbred	10.4	83.4	10.2	87.6	85.5	
190×191 hybrid	9.7	82.1	10.3	87.3	85.9	

^a σ cannot be calculated because the duration of experiment and room humidities involved in individual experiments were somewhat different. The averages presented give only the relative relationships of the two inbreds and their hybrid.

true, it is probable that the apparently constant rate of transpiration attained after an hour's time represents the rate after the stomates have closed. It is this rate which is called in this paper "cuticular transpiration." The curve for #1 inbred in figure 1 resembles somewhat curve B in figure 1 (inset).

Two exceptional types of water loss were occasionally found. An example of each is shown in figure 1 (inset). Curve A is tentatively explained by assuming that the stomates were essentially closed at the beginning of the experiment and remained closed. Curve B is explained by assuming that the stomates

Many of the transpiration curves of the type in figure 1 have been plotted in this semi-log fashion. Since there seems to be no theoretical reason for supposing that lines fitted by the method of least squares, or by any of the other common technical procedures, should be more appropriate than lines sketched by sight, we have used the latter method of drawing in the lines. In nearly every case the fit of such lines seems satisfactorily close. To get more points, one experiment was done in which one leaf was weighed every two minutes. The results are shown in figure 2, curve D.

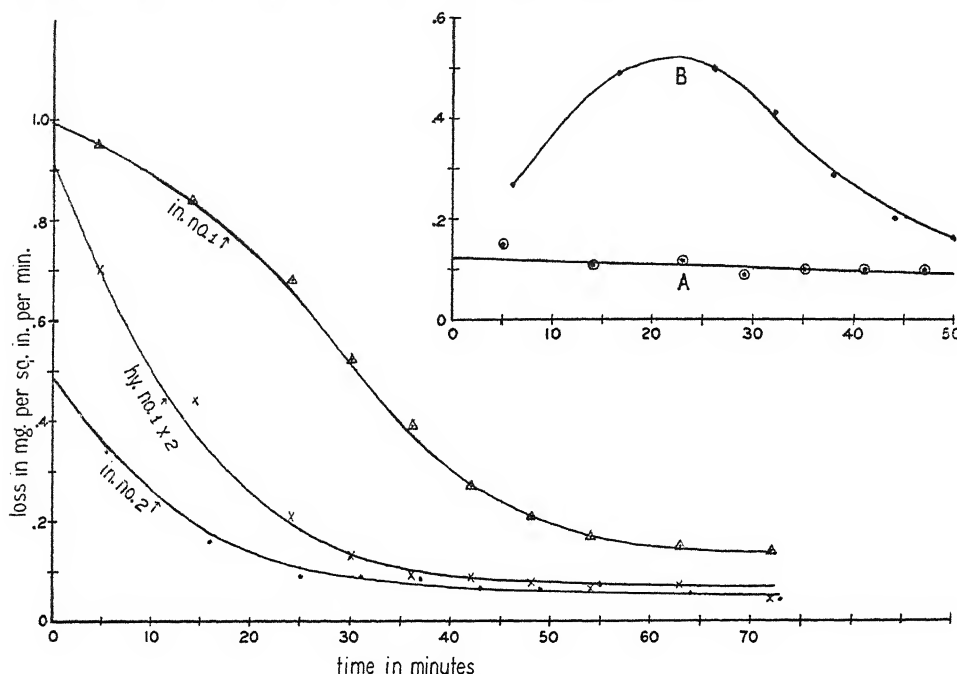


Fig. 1. Graph of data concerning water loss from excised corn leaves. Explanation in text.

were partly closed, that as the epidermal cells began to wilt they relaxed pressure on the guard cells and the stomates opened, later closing again as the guard cells lost turgor (cf. Stålfelt, 1929, 1932).

If the data shown in figure 1 are plotted semi-logarithmically, plotting the logarithm of "stomatal transpiration" ($r-d$) against time, a straight or nearly straight line is obtained. (See fig. 2.) Such a curve is represented by the equation²

$$\log (r-d) = bt + c$$

where t is the time elapsed after the cutting off of the leaf, r is the rate of total transpiration, determined from the difference between two successive weighings, d is the rate of cuticular transpiration, c is the estimated logarithm of stomatal transpiration at $t=0$, and b is the slope of the line. This logarithmic function becomes unsuitable when $(r-d)$ becomes less than one.

² We are indebted to Dr. J. H. Curtiss of the Mathematics Department of Cornell University for help with the mathematics employed in this paper.

The slopes of the curves for the various inbreds and hybrids, insofar as they were graphed, were nearly the same. (The different slopes in fig. 2 are not characteristic of the particular pedigrees.) This means that the decrease in the logarithm of stomatal transpiration was essentially independent of the strain of corn studied; this implies in turn that the speed with which the stomates close is independent of the strain of corn used. It seems unlikely that the stomates of six inbreds and three hybrids should all close at the same relative rates unless the same mechanism be operative in all cases. In the present case the closure of stomates is probably due to the rapid wilting of the entire leaf. It would seem that water loss by evaporation is so rapid that the usual osmotic and imbibitional influences of the guard cells are not given time to act. If this is true, such an experiment of weighing excised leaves can never measure differences in closure of stomates under such adverse conditions as are commonly met with, unless the humidity about the leaves is kept quite high. This was not done in the present experiments.

The water losses from a few excised bean leaves (*Phaseolus vulgaris* L., var. Red Kidney) gave linear graphs when plotted in the above-described semi-log fashion. Furthermore, the data given by Stålfelt (1932) for an excised birch leaf and by Paltridge and Mair (1936) for whole plants of a number of different Australian grasses seem also to fit a semi-logarithmic straight line of the type mentioned. The same is true for data given by Pfeiderer (1933) concerning water loss from excised shoots of a number of plants, including *Stachys germanica*, *Picea excelsa*, *Buxus sempervirens*, and *Sambucus nigra*.

Pfeiderer's (1933) and Stålfelt's data (1932) for width of stomates also fall into a straight line with semi-log plotting. Their data for stomate width and "stomatal transpiration," plotted against each other on log-log graph paper, fall in a straight line.

These similarities of stomatal closure and "stomatal transpiration" strengthen the assumption that the initial rapid decrease in rate of water loss is due to closure of stomates. The diverse sorts of plants that show a semi-logarithmic relationship between stomatal transpiration and time, when all external sources of water supply are removed, make it seem probable that such a relationship is very general. However, it will presumably appear only when parts of plants are excised, or when entire plants are pulled up.

SUMMARY

Excised leaves of various inbred and hybrid corn seedlings were weighed repeatedly until the rate of water loss became constant. The various statistics calculated from the data so obtained were independent of the pedigree. Thus, no real differences between strains with regard to water loss were found.

The data obtained seem to fall in a straight line curve when the logarithm of stomatal transpiration is plotted against time. It is concluded that such a semi-log relationship between water loss and time is general for excised leaves and for living plants unable to absorb water.

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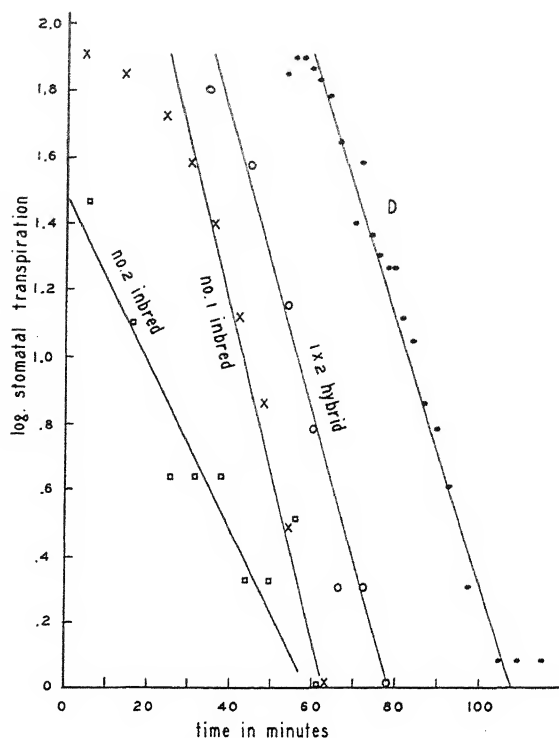


Fig. 2. Semi-logarithmic graph of data on water loss from excised corn leaves. The 1×2 hybrid curve has been moved to the right 30 units, and curve D has been moved to the right 50 units; this was done to avoid undue juxtaposition of the curves. The true figures for stomatal transpiration have been multiplied by 100 in order to cause all of the logarithms to be positive.

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SOME EFFECTS OF PHOTOPERIOD ON LEAF GROWTH¹

Irene H. Stuckey

It is generally recognized that, irrespective of the flowering response of plants grown with different photoperiods, vegetative growth is most prolific under exposures of sixteen to eighteen hours (Austin, 1935; Weaver and Himmel, 1929; Doroshenko, 1927). If the critical period for a long-day plant is reached and flowering occurs, the weight may be less than that of a short-day vegetative plant, but usually the plants grown with long photoperiods are consistently larger.

In their classical papers on photoperiodism, Garner and Allard (1923) recognized the influence of day-length on the vegetative development of plants as well as on flowering, but, with few exceptions, most of the investigations following these have been

the inhibition of stem elongation as the simplest evidence of photoperiodism.

The results reported here developed from the observation that several species of grasses growing with eight- and sixteen-hour photoperiods showed striking differences in habit of growth. The plants exposed to an eight-hour photoperiod tended to be prostrate in their growth habit. The plants which received a sixteen-hour photoperiod had relatively few tillers, and the habit of growth was strongly ascending. Both the sheaths and the blades of the leaves were much longer in proportion to the width than those of plants growing with the shorter photoperiod. An early stage during the growth of orchard grass is shown in figure 1.

There was some speculation as to whether these differences in habit had any recognizable anatomical basis, and if so, whether it could be measured, and an investigation along these lines was begun. The points to be considered were the relation between leaf size and cell number and differences among the cells. Were the leaves longer because they contained more cells or larger cells? Were the leaves of the plants growing under the long day more erect because they contained more mechanical tissue, or was the mechanical tissue more rigid than that in the plants exposed to the short day?

EXPERIMENTAL.—Materials and methods.—Of the several species of grasses in which this phenomenon was noticed, orchard grass (*Dactylis glomerata* L.) seemed best adapted for an anatomical study. The leaves are long and relatively wide. It grows readily under greenhouse conditions, and the change from the vegetative state to flowering can be detected by the elongation of the first internode even before flower primordia can be found with dissection.

Commercial seed of orchard grass was planted in fertile soil in greenhouse beds and, after several weeks, the plants were thinned to the desired spacing. The first series of plants was grown from October through December and the second from January through June. Four beds were used. Two beds received eight hours of daylight and were shaded with black sateen curtains during the remainder of the day. The other two beds were given supplementary light which increased the photoperiod to sixteen hours. On days when the intensity of daylight was very low, all beds received supplementary mazda light during the daylight period.

Leaf samples were collected approximately every two weeks, the youngest fully expanded leaf from several plants being taken each time. The leaves were considered fully developed when the base of the blade had emerged from the sheath of the next older leaf. The leaves were boiled in 70 per cent alcohol until the chlorophyll was extracted and then stored in fresh 70 per cent alcohol until needed.

To study the lengths of the cells in the leaves, macerations from the midribs were made according



Fig. 1. Orchard grass growing with two different photoperiods. The plant on the left was exposed to a sixteen-hour day, the plant on the right to an eight-hour day, and the one in the center to natural day length. The photograph was made November 20, 1939, when the plants were four weeks old.

concerned with the induction or inhibition of flower primordia. Murneek (1940) has distinguished between photoperiodic effects on the vegetative growth and flowering of *Rudbeckia*. Went (1941) reported the effects of light on stem and leaf growth; he cites

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to the method of Jeffery as described by Johansen (1940). The midribs were dissected from the laminae and macerated at 58°C. in a mixture of dilute chromic and nitric acids, the time and strength of the solution varying with the material. The time required for successful maceration of midribs from plants exposed to the sixteen-hour day was usually twice as long as for those collected at the same time from plants growing with the eight-hour day. When the maceration had progressed until the cells would separate readily and yet not disintegrate, the material was washed thoroughly with water. Small portions were stained with safranin, then destained with 95 per cent alcohol, cleared in diaphane solvent and finally mounted in diaphane. This is a relatively simple method for obtaining large numbers of isolated cells which can be measured easily with a calibrated ocular micrometer.

Since these macerations gave only longitudinal views of the midribs, cross sections were made from some of the same material. Short lengths of the midribs were embedded in paraffin after being run through the n-butyl alcohol series. The sections were stained with Heidenhain's hematoxylin and safranin.

Epidermal cells were counted directly on known areas of tissue, and estimates of the number of cells in the leaf were derived from these counts.

At intervals, the length and width of the longest leaf from fifty plants were determined, and the numbers of tillers on the plants were counted. The color of the plants grown with the eight-hour photoperiod seemed to be a more intense green than those grown with the long day, but no attempts were made to measure any differences.

Results.—Examination of the macerations showed that the mesophyll cells were the same size in all the preparations, and no measurements of these cells were taken. Most of the annular vessel elements were torn, but the reticulate elements were intact and easily measured. Since the end wall perforations were distinctive, broken segments of vessel elements could be recognized and discarded. The lengths of the vessel elements from both series are shown in table 1. There were greater individual variations in the lengths of the fibers, but a larger number was measured to compensate for this as shown in table 2. The differences in length of the fibers were even more significant than those of the vessel elements. The dif-

TABLE 1. *Average length of vessel elements.*

Date	Number of cells measured	Mean length (microns)	Difference D, long-short	Standard error of mean difference, E_d	D/E_d^a
November 20, 1939:					
Long day	94	565
Short day	44	395	170	± 23.33	7.29
December 3, 1939:					
Long day	77	759
Short day	79	496	263	± 36.58	7.20
December 18, 1939:					
Long day	71	711
Short day	37	414	297	± 28.93	10.24
December 25, 1939:					
Long day	128	703
Short day	41	587	116	± 24.61	4.73
January 8, 1940:					
Long day	84	545
Short day	53	510	34	± 23.73	1.45
March 25, 1940:					
Long day	179	482
Short day	141	325	157	± 10.68	14.71
April 18, 1940:					
Long day	182	638
Short day	139	358	280	± 16.88	16.59
May 2, 1940:					
Long day	179	580
Short day	198	423	157	± 14.83	10.61
May 16, 1940:					
Long day	175	655
Short day	156	626	29	± 17.03	1.68
May 31, 1940:					
Long day	192	716
Short day	196	676	40	± 16.06	2.49
June 22, 1940:					
Long day	150	648
Short day	196	679	-31	± 17.38	-1.79

^a D/E_d must be greater than 2 to be significant.

TABLE 2. *Average length of fibers.*

Date	Number of cells measured	Mean length (microns)	Difference D, long-short	Standard error of mean difference, E_d	D/E_d^a
November 20, 1939:					
Long day	192	571
Short day	153	422	148	± 15.39	9.64
December 3, 1939:					
Long day	102	969
Short day	101	607	361	± 51.90	6.96
December 18, 1939:					
Long day	175	948
Short day	35	624	325	± 41.90	7.75
December 25, 1939:					
Long day	186	865
Short day	178	579	286	± 29.70	9.61
January 8, 1940:					
Long day	200	677
Short day	237	502	174	± 15.33	11.37
March 25, 1940:					
Long day	199	497
Short day	200	431	66	± 15.36	4.60
April 18, 1940:					
Long day	199	696
Short day	199	569	127	± 18.17	6.99
May 2, 1940:					
Long day	200	823
Short day	196	477	346	± 20.98	16.48
May 16, 1940:					
Long day	202	989
Short day	226	568	421	± 23.98	16.18
May 31, 1940:					
Long day	198	1,033
Short day	199	726	307	± 18.19	16.87
June 22, 1940:					
Long day	190	976
Short day	199	746	230	± 26.94	8.53

^a D/E_d must be greater than 2 to be significant.

ferences both in vessel elements and fibers were greatest when the plants were growing most rapidly, tending to level off toward the end of the growing period.

A parallel experiment was performed with Colonial bent (*Agrostis tenuis* Sibth.). The results were essentially the same as those shown on tables 1 and 2 and are not included here.

The estimate of the numbers of epidermal cells in the leaf, based on counts from known areas of the leaves (table 3), indicate that here also the length of the cells in the leaf was increased rather than the number of cells. The width of the leaves increased very little in proportion to the length (table 4). It was not determined whether a greater number of mesophyll cells was present in the leaves from the plants under the sixteen-hour photoperiod, or whether the intercellular spaces were larger.

Fibers seemed to be more numerous in the preparations made from the long-day plants. Cross sections of the midribs showed that, while there was a small increase in numbers of the fibers as is shown in the second row of fibers on one side of the bundle (fig. 2), the apparent increase is due to the greater

length of fibers. Many of the fibers were long and twisted and could not be measured easily. The most noticeable difference in the cross sections of the midribs was the striking wall thickness of the fibers grown with the long photoperiod. The phloem in the leaf from the long-day plant (fig. 2) appeared less mature than that from the short-day plant; yet the fiber walls were much thicker. This wall thickening probably accounts for much of the increase in rigidity of the leaves of the long-day plants.

Murneek (1940) has observed that in *Rudbeckia*, the position of the leaves changes from horizontal to vertical after induction of flower primordia has taken place, but none of the orchard grass plants used here ever changed to the flowering condition. At the end of the experiment neither elongated internodes nor primordia were found on any of the plants.

DISCUSSION.—Tincker (1925), using clonal stock, found that orchard grass responded as a long-day plant. He presented evidence that the treatment of plants during one season influenced the flowering response the following year. Sprague (1941) more recently observed that none of the clonal material he was using headed under normal winter days nine to

ten hours long. Several clones headed and flowered under a twelve-hour day, but the type of growth was very decumbent, and heading was three or four weeks later than that obtained under a sixteen-hour day. While not all of the clones headed with the sixteen-hour day, a considerably larger number produced erect normal heads. Sprague believes that the treatment of an orchard grass plant for six to ten

of photoperiod on cell length was not further complicated by the effects of photoperiod in inducing flower primordia. All the plants used here were vegetative.

The effects of light on the growth of leaves have been studied by several workers. Gregory (1921) considered that the area of the leaf surface is closely related to the intensity of incident radiation. Went

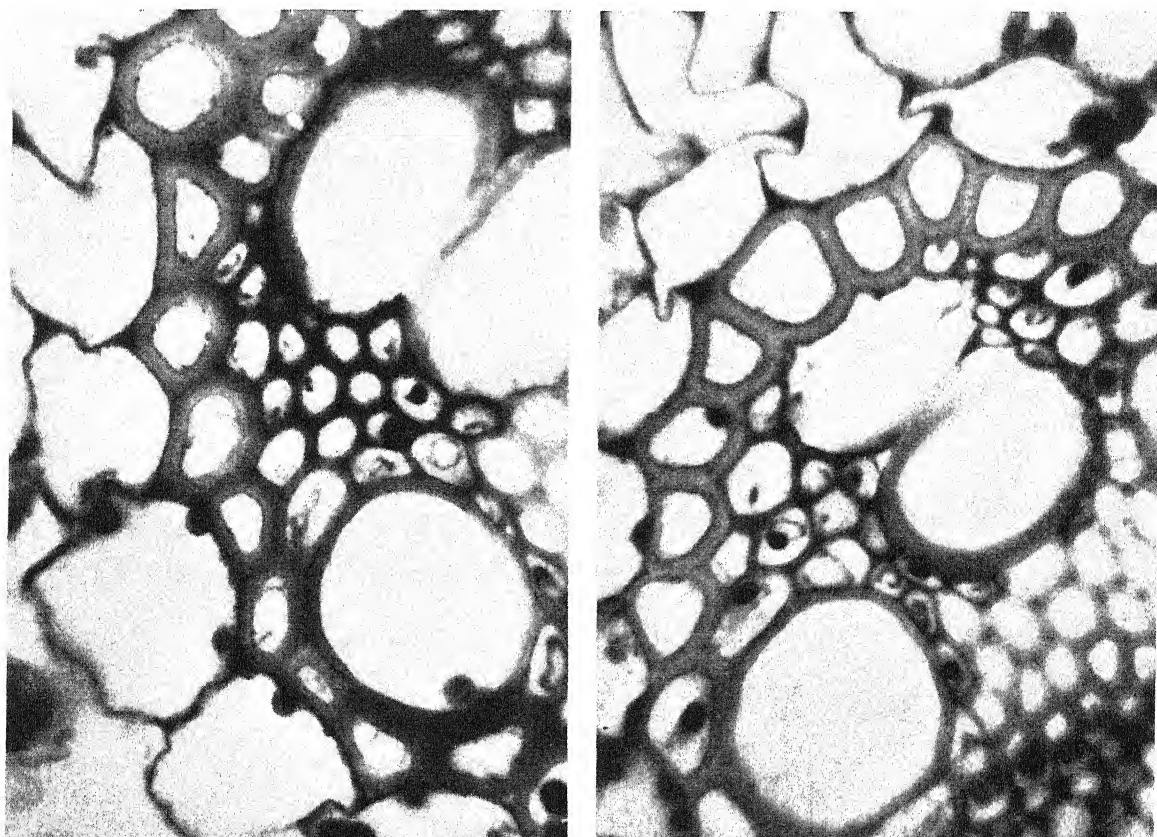


Fig. 2. The section on the left was taken from the midrib of a leaf grown with a sixteen-hour day, that on the right from one exposed to an eight-hour day. The plants were collected April 18, 1940.

weeks prior to a long photoperiod conditions the response of the plant to the photoperiod.

The writer has found, when using seedlings in experiments similar to those just described, that eventually a small percentage of the plants flowered under an eight-hour day if the experiments were continued for eight months, but the critical photoperiod seemed to be no more than twelve hours. Formation of flower primordia was never induced with a sixteen-hour photoperiod during the course of these experiments. It was not determined whether the contradictory results obtained with the seedlings by the writer were due to the strain of orchard grass used, or whether the response of the clones used by other workers was influenced by the environmental conditions to which they were exposed prior to the photoperiod experiments. Under these conditions the assumption can be made that the study of the influence

(1941) found that within the range of $1/10,000$ ergs/cm.² each day the leaf length is proportional to the log of the amount of energy supplied. With more light the effect falls off, as might be expected. Undoubtedly at lower intensities the incident radiation is the limiting factor in leaf growth, but for most plants photoperiods from sixteen to eighteen hours give maximum vegetative growth, longer periods resulting in injury. The unequal growth of the orchard grass leaves cannot be explained as being caused by differences in the total radiation. The evidence strongly suggests that the differences in leaf size are responses to the differences in photoperiods. Tincker (1928) reported that in early spring the leaves of orchard grass had short sheaths, and, as the days became longer in May and June, leaves with longer sheaths were produced.

TABLE 3. *Number of epidermal cells in leaf.*

Date:	March 25, 1940		May 2, 1940		June 22, 1940	
Treatment	Long day	Short day	Long day	Short day	Long day	Short day
Length of leaf	137 mm.	67 mm.	450 mm.	195 mm.	744 mm.	450 mm.
Average number cells/sq. mm.:						
Top of leaf	185.9	139.6	144.9	126.3	111.9	124.6
Middle of leaf	75.6	166.2	64.9	102.2	65.4	64.3
Bottom of leaf	74.9	71.1	55.5	106.9	38.9	51.7
Estimated number of cells in entire leaf	15,239	7,290	35,295	21,801	53,618	36,090
Estimated number of cells in length of leaf	1,899	845	4,020	2,756	5,664	4,020

TABLE 4. *Average length of longest leaf from fifty plants (inches).*

Date:	Nov. 16, 1939	Nov. 27, 1939	Dec. 25, 1939	Jan. 8, 1940	July 1, 1940
Long day	13	14	24	27	48
Short day	4	4	7	8	31

Investigations of the influence of photoperiod on the anatomy of leaves have been conducted by Deats (1925), Pfeiffer (1926) and Tincker (1928), but they were incidental to a study of the anatomy of the stems. These workers found differences in leaf thickness which were due to elongation of the palisade cells, but environmental factors other than photoperiod seemed to play some part, since results with the same species were not always consistent. No references were found including studies of midribs of leaves of any species. Redington (1929) measured the length of leaves of *Pelargonium*, *Polygonum* and *Chrysanthemum* growing with different photoperiods, entirely with artificial light. In every case, the laminae were largest with the sixteen-hour photoperiod. In no instances were any cell measurements made.

Most of the anatomical studies of the effect of photoperiod have dealt with stems almost exclusively, and insofar as only primary tissues are involved, the results may be considered applicable to these same tissues in leaves. Redington (1929) found that the linear dimensions of the endodermal cells in the stems of flax, hemp, hop, salvia, hibiscus, and cotton varied inversely with the light period. His photographs show that the use of artificial light alone caused marked etiolation, especially in the plants growing with the eight-hour photoperiod.

Doroshenko (1927) found that within limits the cell size in stems of flax became smaller as the day length was decreased, then below a certain level began to increase again. In some strains of wheat, the leaves were more numerous and longer under a nine-hour day as compared with a twelve-hour day or normal summer day length. However, his results can be attributed to a difference between vegetative and reproductive growth rather than the effect of photoperiod on vegetative growth.

A striking difference noticed in the midribs of the orchard grass leaves, which is comparable to results reported by others in stems, was the marked thickening of the walls of the sclerenchyma of the bundle sheaths. Such wall thickening was noted in the stems of several species of perennial plants by Redington and Priestley (1925). The thinness of the walls of fibers grown with an eight-hour light period was especially striking. Deats (1925) observed that in pepper and tomato plants the walls of the "bast" were thicker, and that in pepper the walls of the xylem also were thicker, in the plants growing with the long photoperiod. In these plants, the epidermal cells of the stems were longest with the long photoperiod.

At present no theories are presented which will explain the differences found in cell length. More carefully controlled conditions are necessary to determine whether the difference found was caused primarily by photoperiod or by some other factor.

SUMMARY

Orchard grass seedlings grown in eight- and sixteen-hour photoperiods were very different in size and habit of the plants. Plants grown under the long photoperiod were characterized by longer leaves and more erect growth habit. These differences were correlated with the following differences in their anatomy:

1. The lengths of the vessel elements, of the fibers and of the epidermal cells, varied directly with total organ size, indicating that leaf length differences were due to cell size and not cell number, at least with respect to these cell types.

2. The size of the mesophyll cells remained the same; hence size differences in this tissue may be due either to differences in cell number or in the size of the intercellular spaces.

3. The walls of the fibers were noticeably thicker

in long-day plants which is probably correlated with their more upright habit, but no such difference was observed in the walls of vessel elements.

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FACTORS CONTROLLING SPORANGIAL TYPE IN THRAUSTOTHECA PRIMOACHLYA AND DICTYUCHUS ACHLYOIDES. I¹

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THE GENERA of the Saprolegniaceae are distinguished from one another by their methods of asexual reproduction, i.e., by the characteristics of the sporangium, the type of spore discharge, and the extent of the swarmer cycle. Although originally but one method of sporangium dehiscence was known for each genus, three species are now recognized in which two methods are present, each of which is representative of a different genus. In *Thraustotheca primoachlya* (Coker and Couch, 1924), the spores of the initially formed sporangia, after emergence through an apical papilla, become arranged in a rather irregular hollow sphere at the sporangial apex as in the genus *Achlya*, whereas those of the later sporangia are liberated through an irregular rupture of the outer sporangium wall as in the genus *Thraustotheca*. In *Dictyuchus achlyoides* (Coker, 1927), the sporangia are at first of the *Achlya* type, but are replaced subsequently by those characteristic of the genus *Dictyuchus*, in that they have a "cell-net" structure with the zoospores emerging separately from individual compartments. In *Brevilegnia bispora* (Couch, 1927), which may better be called

Thraustotheca bispora (Salvin, 1942), the mycelium first gives rise to sporangia characteristic of the genus *Achlya*, but later to those characteristic of the subgenus *Brevilegnia*, in which the sporangiospores are dispersed by an irregular rupture of a very thin sporangial wall. These two latter forms will for convenience be termed "achlyoid" and "brevilegnoid" respectively.

Although these transformations in the methods of sporangial dehiscence have been carefully described, little is known about the basic causes. The assumption has been that the change is purely of genetic origin, although no experimental work has been reported to establish this belief. However, if the environment does produce, partly or wholly, the change in the sporangia, not only may the true taxonomic position of the species then be clarified, but also new facts on the relationship of the genera of the Saprolegniaceae may be forthcoming. Accordingly, the writer conducted investigations on *Thraustotheca primoachlya* and *Dictyuchus achlyoides* in order to determine the exact basis for the appearance of two distinct types of sporangia on the same mycelium in each of these species.

MATERIALS.—Most of the experimental work was conducted on two species: (1) *T. primoachlya*, isolated from soil along the Charles River, Cambridge, Massachusetts, by J. R. Raper; and (2) *D. achlyoides*, isolated by Ralph Emerson from mud obtained

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from a swamp in Kampala, Uganda, Africa. A few experiments were also done on other species.

EXPERIMENTS AND RESULTS.—*Normal life cycles.*—When *T. primoachlya* was grown on a hemp seed in 25 cc. of redistilled water at room temperature (22° to 23°C.), all the sporangia formed during the first three days were achlyoid. Subsequently, although many sporangia still liberated their spores as in *Achlya*, more and more began to dehiscence in the thraustothecoid manner until, from the fifth day on, all the sporangia were of the latter type.

When *D. achlyoides* was grown under the above conditions, both achlyoid and dictyuchoid sporangia were produced in large numbers during the first two days. Thereafter, the quantity of newly formed achlyoid sporangia rapidly dwindled, until by the end of the third day only the "cell-net" type was developed.

If transformations in the sporangia are due to changes in the environment, three possible influential factors must be considered: (1) a decrease in the oxygen tension; (2) the presence of a critical hydrogen ion concentration; or (3) the accumulation of an active staling substance.

Investigations on the influence of a decrease in oxygen tension.—Since the fungus mycelium is aerobic, it requires oxygen in amounts sufficient to permit the normal functioning of the metabolic processes. If this oxygen were to be consumed by the hyphal mass from the aqueous medium at a rate greater than that at which it is being replenished from the external air, a concentration might result which would disrupt the internal processes to such a degree that the morphology and behavior of the sporangia become altered. If, however, a continuous supply of oxygen is available, then no change in the sporangia should occur.

One hemp seed inoculated with *T. primoachlya* was suspended in a microaquarium in 25 cc. of water through which air was being bubbled, and another similarly inoculated was placed under the same conditions, except that the air was obtained by diffusion through the surface of the water. The mycelium in the vigorously aerated water formed achlyoid sporangia at first, just as in the control, and for about the same length of time, although comparatively few sporangia were formed at all. Evidently, therefore, a decrease in oxygen tension is not the factor responsible for the change in sporangial type.

Investigations on the influence of hydrogen ion concentration.—As the mycelium of *T. primoachlya* developed, the 25 cc. of aqueous medium gradually became more alkaline, the hydrogen ion concentration decreasing from pH 5.5 on the first day of growth to pH 7.3 on the fourteenth, with the change in sporangial type from achlyoid to thraustothecoid occurring at pH 6.3 to 6.7. These results suggest that the presence of a critical hydrogen ion concentration in the external medium may induce the formation of thraustothecoid sporangia exclusively.

This, however, was demonstrated to be untrue. When *T. primoachlya* and *D. achlyoides* were each

grown in 25 cc. of M/50 phosphate-buffer solutions of pH 3.5 to 8.6, achlyoid sporangia were the first to appear in all the cultures. If the alkalinity of the water did influence the nature of the sporangia, only thraustothecoid would have developed in the solutions with a pH above 6.5 and only achlyoid in those below about 6.5. Hence, the conclusion can be drawn that the change in the sporangia is not associated with a decrease in hydrogen ion concentration.

Investigations on the influence of staling material.—Since the products of mycelial metabolism are excreted into the surrounding medium, there is the possibility that the attainment of a threshold concentration of a staling substance may influence the behavior of the sporangia. In order to prevent the accumulation of any such active staling substance and, therefore, to inhibit the formation of thraustothecoid sporangia, a mycelium of *T. primoachlya* was grown on a hemp seed in 25 cc. of glass-distilled water which was constantly being renewed by a perfusion apparatus (Raper, 1939). Here again the sporangial cycle was typical, with great numbers of achlyoid sporangia appearing first and then being succeeded by thraustothecoid. When similar experiments were carried out with tap water, ordinary distilled, glass-distilled plus salts, and spring water, similar results were obtained.

However, when *T. primoachlya* grew on a hemp seed in 25 cc. of water in which another colony of the same organism had previously developed for two weeks, only thraustothecoid sporangia were formed. Some substance evidently had been excreted which inhibited the production of achlyoid sporangia.

That this active staling principle was not specific to the mycelium of *T. primoachlya* was shown when similar experiments were conducted with the staling substances from other species. After *Achlya flagellata* Coker, *Aphanomyces laevis* deBary, *D. achlyoides*, *Dictyuchus* sp., *Saprolegnia* sp., *Thraustotheca clavata* (deBary) Humphrey, *T. primoachlya*, and *T. unispalma* Coker and Braxton had each been grown on twenty hemp seeds in 25 cc. of water at 25°C. for ten days, the water from each species was drawn through a Chamberland filter and used again for the growth of *T. primoachlya* on a single hemp seed.

Four types of responses by the mycelium of *T. primoachlya* were discernible: (1) formation of many sporangia of both achlyoid and thraustothecoid types; (2) development of only thraustothecoid sporangia, with complete suppression of the achlyoid; (3) extensive mycelial growth, with a tendency toward suppression of all sporangia, but with thraustothecoid sporangia predominating among the few formed; and (4) inhibition of both sporangium formation and vegetative growth. The extent of the influence of the different species was related to the rate of their mycelial growth (fig. 1), since the species with extremely rapid growth (e.g., *Saprolegnia* sp.) inhibited the mycelial and sporangial development of *T. primoachlya*; those with intermediate rates (e.g., *T. clavata*) inhibited completely the development of

achlyoid sporangia, and partially that of thraustothecoid sporangia, but actually increased vegetative growth; and those with low rates (*e.g.*, *Aphanomyces laevis*) either inhibited slightly the development of achlyoid sporangia or had no influence whatsoever.

Many genera evidently produce a substance, or substances, which, if present in the proper amount, influence the type and number of sporangia of *T. primoachlya*. As the species with the highest growth rate has the greatest influence on the sporangia of *T. primoachlya*, this same species probably produces the most staling material.

When a colony of *T. primoachlya* is developing under normal conditions, this active principle is probably excreted continually into the water, in relatively small quantities of which a definite threshold is attained that results in the transformation in the type of sporangium. The time necessary for the appearance of the threshold concentration should, therefore, increase in proportion to the volume of water present.

To test this concept, single hemp seeds inoculated with *D. achlyoides* and *T. primoachlya* were placed in quantities of water from 1 cc. to 3,000 cc. and the time recorded when achlyoid sporangia ceased being developed. The results were clear-cut and in support

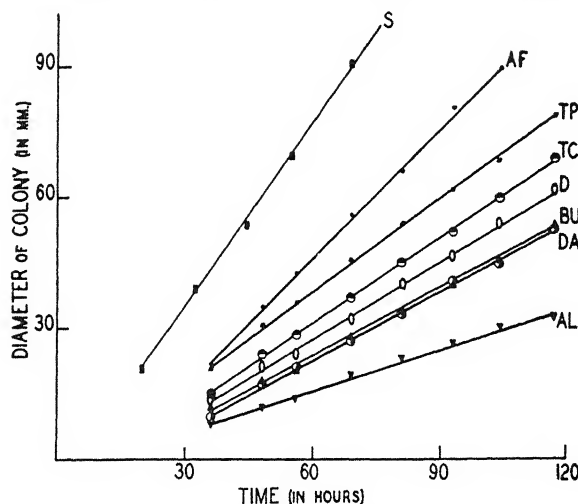


Fig. 1. Graph, showing the rate of mycelial growth of eight different species of the Saprolegniaceae. (AF, *Achlya flagellata*; AL, *Aphanomyces laevis*; BU, *Thraustotheca unispersma*; D, *Dictyuchus* sp.; DA, *Dictyuchus achlyoides*; S, *Saprolegnia* sp.; TC, *Thraustotheca clavata*; TP, *Thraustotheca primoachlya*.)

of the above view (fig. 2). In *T. primoachlya*, no achlyoid sporangia at all were apparent in either 1 cc. or 2 cc. of water. In 5 cc., some achlyoid sporangia were formed, but these were entirely replaced by thraustothecoid in 24 hours. In 10 cc. of water, achlyoid sporangia no longer were developed after about 70 hours; in 25 cc., after 105 hours; in 50 cc., after 144 hours; in 100 cc., after 270 hours; etc. Similar results were obtained for *D. achlyoides*,

although in this species achlyoid sporangia were always accompanied by dictyuchoid and were formed for comparatively briefer periods in the corresponding volumes of water.

To establish a broader basis for the concept that a threshold concentration of a staling material inhibits the development of achlyoid sporangia, a colony of *T. primoachlya* on a hemp seed was grown in

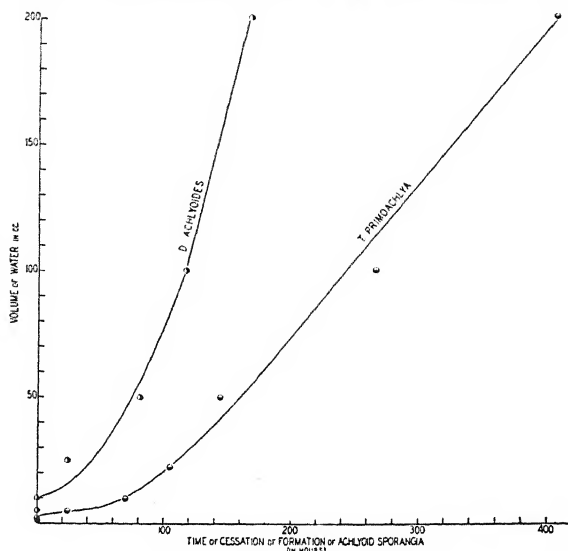


Fig. 2. Graph, illustrating the relation between (1) the volume of water in the culture, and (2) the time of cessation of formation of achlyoid sporangia.

1 cc. of water for 24 hours, during which time only thraustothecoid sporangia appeared. When the mycelium was transferred to 40 cc. of water and examined twelve hours later, many achlyoid sporangia were apparent. On removal to 1 cc. of water, the hyphal mass again formed only thraustothecoid sporangia.

These facts seem to justify the conclusion that the mycelium gradually excretes an active substance which inhibits the formation of achlyoid sporangia when a threshold concentration is reached. Although the active principle is apparently present in many species, its exact nature has not been determined as yet.

Relation of the staling substance to oxygen tension.—Preliminary experiments on the influence of aeration on the formation of sporangia in *T. primoachlya* showed that increased amounts of oxygen failed to inhibit the development of thraustothecoid sporangia. However, the hyphae of *T. primoachlya* on the surface of 50 cc. of water formed thraustothecoid sporangia at least twenty-four hours before those below the surface. Since these surface hyphae are exposed to a rather high oxygen tension, the rate of formation of the active staling material may conceivably be increased thereby, and as a result thraustothecoid sporangia would appear comparatively early.

That this hypothesis is true is indicated by the following experiment. Mycelia of *T. primoachlya* were grown on single hemp seeds in 200 cc. quantities of water (1) in a large Petri dish 19 cm. in diameter, (2) on the surface in a crystallizing dish 9 cm. in diameter, and (3) at a depth of 4 cm. in a similar crystallizing dish. In (1), achlyoid sporangia were replaced by thraustothecoid after 116 hours; in (2), after 142 hours; but in (3), achlyoid sporangia were still being formed 350 hours after the time of inoculation. These results do indicate that a high oxygen tension hastens the rate of formation of the active staling substance.

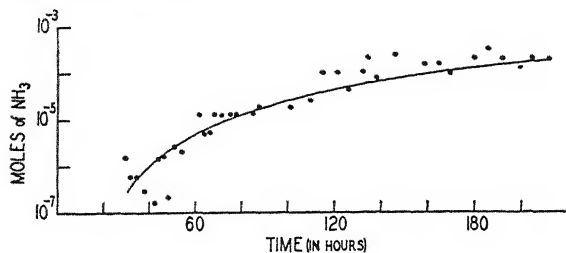


Fig. 3. Graph, showing the rate of production of ammonia by *Thraustotheca primoachlya*.

Investigations on the ammonium ion, or ammonia, as the active staling principle.—Since the water in which cultures of either *T. primoachlya* or *D. achlyoides* were developing gradually increased in alkalinity and contained much ammonia, as indicated by a positive reaction on the addition of Nessler's reagent, it seemed possible that ammonia, or the ammonium ion, initiated the change in sporangia. Accordingly, experiments were conducted to cast further light on this point.

In order to determine at what concentration of ammonia the change in sporangia occurred, the rate and extent of ammonia formation were measured. Fifty inoculations of *T. primoachlya* on single hemp seeds were made in dishes each with 25 cc. of water and grown at 25°C. for over 200 hours. Every few hours, 5 cc. were removed from successive dishes and the amount of ammonia determined by the addition of two to three drops of Nessler's reagent and the subsequent comparison with a standard solution of ammonium hydroxide in a colorimeter. The concentration of ammonia increased from about $5 \cdot 10^{-7}$ M. at 40 hours to over $5 \cdot 10^{-4}$ M. at 220 hours, with the change in sporangia appearing at about $5 \cdot 10^{-5}$ M. (fig. 3).

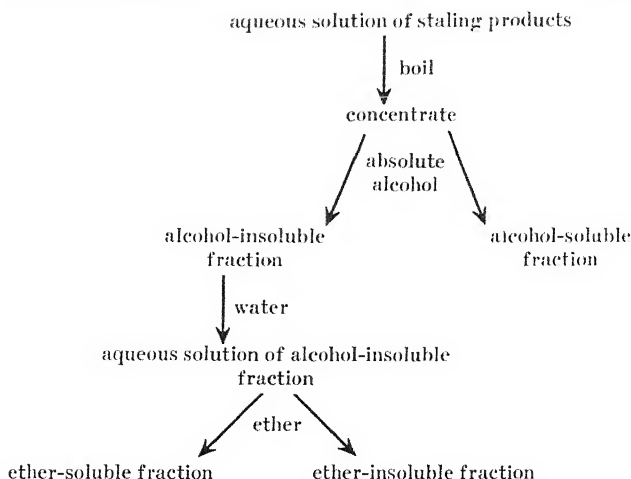
With the concentration established at which the change in sporangial type occurred, both *T. primoachlya* and *D. achlyoides* were grown in solutions of several ammonium compounds and examined for indications of the inhibition of achlyoid sporangia. To exclude the possibility that the anion influences the development of the sporangia, mycelia of the two species were also grown in solutions of compounds which had the same anions as the ammonium mole-

TABLE 1. Table to show types of sporangia formed in solutions of ammonium compounds.^a

Concentration:	10 ⁻⁶	10 ⁻⁵	6 × 10 ⁻⁶	8 × 10 ⁻⁶	9 × 10 ⁻⁶	10 ⁻⁵	2 × 10 ⁻⁵	3 × 10 ⁻⁵	4 × 10 ⁻⁵	5 × 10 ⁻⁵	6 × 10 ⁻⁵	8 × 10 ⁻⁵	10 ⁻⁴	10 ⁻³	5 × 10 ⁻³	6 × 10 ⁻³	8 × 10 ⁻³	10 ⁻²
Compound:	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.	M.
<i>Thraustotheca primoachlya</i>																		
NH ₄ Cl	A ^a	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0
KCl	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
(NH ₄) ₂ SO ₄	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0
Na ₂ SO ₄	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0
(NH ₄) ₂ CO ₃	..	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0
Na ₂ CO ₃	..	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0
NH ₄ OH	..	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0
<i>Dictyuchus achlyoides</i>																		
NH ₄ Cl	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	0
KCl	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	0
(NH ₄) ₂ SO ₄	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	0
Na ₂ SO ₄	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	0
(NH ₄) ₂ CO ₃	..	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	0
Na ₂ CO ₃	..	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	0
NH ₄ OH	..	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	A+D	0

^a A, achlyoid sporangia; D, dictyuchoid sporangia; T, thraustothecoid sporangia; A+D, mixtures of achlyoid and dictyuchoid sporangia; A+T, mixtures of achlyoid and thraustothecoid sporangia; 0, no sporangia.

Diagram 1. Diagram to illustrate the process of obtaining the several fractions of staling material.



cules but different cations. The results are summarized in the accompanying table (table 1).

In the first place, it is clear that three of the four ammonium compounds did influence the formation of sporangia in *T. primoachlya*, and one of the four had such an effect in *D. achlyoides*, in contrast to the sodium and potassium compounds, which had no visible effects whatsoever. On the other hand, the significance of the rôle of ammonia in the transformation of sporangia is greatly lessened in view of (a) the lack of definite correlation between the concentration of the (NH_4) ion and the formation of a particular sporangial type and (b) the frequent appearance of abnormal vegetative growth. For example, in *T. primoachlya*, only in some of the solutions of ammonium sulfate did complete inhibition of achlyoid sporangia appear, although in several of the ammonium chloride and ammonium carbonate solutions the initially formed sporangia were both achlyoid and thraustothecoid. The inhibition of the development of achlyoid sporangia did not take place at the same concentration of ammonia, the variation being from 0.042 grams of ammonia per liter in the ammonium chloride solution to less than 0.003 grams per liter in the ammonium carbonate solution. These concentrations of ammonia do not agree closely with the one occurring when the transition in sporangial type appears in normal cultures, namely, 0.009 gram per liter. In addition, as the solutions with increased concentrations of ammonia were tested, the vegetative mycelium became abnormal, as characterized by stunted growth and gnarled hyphae, in several cases even before there was any noticeable effect on the sporangia.

These results indicate that ammonia, although produced by many species as a byproduct of normal metabolic processes, is not the principal substance that causes the change in the sporangia, although possibly it does function in some minor rôle.

Investigations on the properties of the staling material.—With the conclusion that the ammonia in the staling material was not in itself the active sub-

stance that induced the transition from achlyoid to either thraustothecoid or dictyuchoid sporangia, direct analysis of the staling products of *T. primoachlya* was attempted. Relatively large quantities of the substance were obtained in the following manner. After the organism had grown in a solution of 1 per cent gelatin, 0.5 per cent peptone, and 0.5 per cent dextrose for about a month, the mycelial mass was thoroughly washed in sterile water in order to remove all adherent nutrient and allowed to develop in glass-redistilled water for an additional three weeks. The resultant solution was then drawn through a Chamberland (porcelain) filter in order to eliminate spores, bits of hyphae, and particulate matter. Since no ingredient of the liquid boiled at less than 100°C . and since the staling principle was not destroyed by several hours of heating at 100°C ., the excess water was slowly evaporated through a Hempel fractionating column without apparent loss of, or change in the staling material. During this condensation, the solution gradually assumed a color first of golden yellow and then of grayish brown.

This concentrate from a liter of water in which the mycelium from 12,000 cc. of nutrient solution had been present for three weeks completely inhibited the development of achlyoid sporangia. The concentrate was still effective in dilutions up to 250 times. The staling material was also active after exposure to a temperature of 250°F . and a pressure of 15 pounds per square inch for 45 minutes, but not after treatment with either strong acid or alkali. In addition, it was very soluble in water, very slightly soluble in ethyl alcohol, and insoluble in ether.

The separation of some of the components of the staling material produced very interesting results. On the addition of 250 cc. of absolute ethyl alcohol to a 10 cc. concentrate of staling material, an extremely fine, grayish-white precipitate appeared, which was then separated from the otherwise clear, pale amber alcohol solution by centrifugation and dissolved in 10 cc. of water. Whereas, after the evaporation of the alcohol, the extract was tested di-

rectly, the aqueous solution of the precipitate, in contrast, was further subjected to fifteen successive extractions of 50 cc. volumes of ether. The resulting residue was dark brown in color, in contrast to the extract, which, after the boiling off of the ether, was colorless (cf. diagram 1).

a. *Alcohol-soluble fraction*.—When *T. primoachlya* was grown on a hemp seed in either 25 cc. or 50 cc. of an aqueous solution of the alcohol-soluble fraction, the vegetative growth, if present at all, was limited to 2 to 3 mm. in diameter and the production of sporangia was completely inhibited (fig. 4 A).

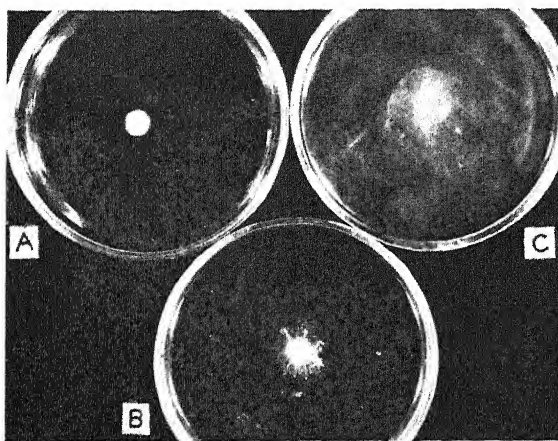


Fig. 4. Photograph, illustrating (A) inhibition of mycelial growth of *Thraustotheca primoachlya* by alcohol-soluble fraction, (B) normal mycelial growth, and (C) increase of mycelial growth by ether-insoluble fraction.

However, when this fraction was partially ashed and subsequently tested in a similar fashion, the mycelium not only developed normally, but also formed moderate numbers of sporangia of the thraustothecoid type exclusively. On complete ashing, both normal growth and normal production of achlyoid sporangia occurred. Therefore, the alcohol-soluble fraction (1) retained some of the achlyoid-sporangium-inhibiting substance, and (2) contained some presumably organic compound which greatly decreased the extent of the vegetative growth.

b. *Ether-soluble fraction*.—When the ether-soluble fraction was tested in a 25 cc. aqueous solution, the mycelial growth and sporangium development of *T. primoachlya* were completely inhibited. When the ash of the fraction was assayed in 25 cc. of water, growth was normal, and achlyoid sporangia developed in relatively large numbers. When acid ether was used for extraction, there was no perceptible change in results from those appearing in neutral ether, although basic ether eliminated the inhibitory effect. Thus, the ether-soluble fraction contains a substance which inhibits vegetative growth very markedly.

c. *Ether-insoluble fraction*.—When 25 cc. of an aqueous solution of the ether-insoluble fraction was assayed, three unusual responses of the mycelium of

T. primoachlya were evident: (a) abnormally extensive vegetative growth (fig. 4 C), (b) inhibition of the formation of achlyoid sporangia, and (c) frequently, a premature development of oogonia. The increased vegetative growth was noticeable not only in the mycelium extending from the hemp seed but also throughout the solution, where the encysted swarmspores gave rise to extremely long, tenuous, branched, and intertwining hyphae. This growth-promoting substance, in addition to the inhibitory one, is not associated with the auxins, since in concentrations from 1:1 to 1:125, it brought about no distinct curvature of decapitated *Avena* coleoptiles. The thraustothecoid sporangia, slightly smaller in size than those formed in pure water, generally appeared in moderate numbers, and not in dense clusters, but intermittently along the hyphae. Although normally oogonia do not appear until the mycelium is about five days old, in several cultures large numbers developed after about 48 hours.

Investigations on the influence of the staling material on other species.—The mycelia of *Achlya dubia* Coker, *A. flagellata*, *D. achlyoides*, *Saprolegnia* sp., *T. clavata*, and *T. unisporma* were grown at 25°C. in 25 cc. aqueous solutions of the ether-soluble and ether-insoluble fractions of the staling material of *T. primoachlya*. In all cases, the solution of the ether-soluble fraction completely inhibited vegetative growth, whereas that of the ether-insoluble fraction augmented it (fig. 5). The solutions of the ether-insoluble fraction affected the formation of sporangia not only of *T. primoachlya* and *D. achlyoides*, in which achlyoid sporangia never developed, but also of *A. flagellata* and *A. dubia*, in which in addition to the characteristic achlyoid sporangia many atypical ones appeared. In the latter category were: (1) those sporangia which never entirely delimited their spore initials and never dehisced; (2) those in which, although spore development was never completed, dehiscence of the papilla occurred and sometimes was followed by partial expulsion of the sporangium contents; (3) those which had irregular masses of spores near their apices instead of the regular hollow spheres; and (4) those which had part of the spore mass expelled and part retained within. Although these thraustothecoid-like sporangia were quite rare in the mycelium of *A. flagellata*, they did definitely occur. The sporangia of *T. clavata* in the latter solutions were normal, except that the great majority were formed on the surface of the medium.

Thus, the active staling principle of *T. primoachlya* inhibited the development of typical achlyoid sporangia not only in that species but also in *A. flagellata*, *A. dubia*, and *D. achlyoides*.

DISCUSSION.—That the staling products of *T. primoachlya* and other species can inhibit the formation of achlyoid sporangia in *T. primoachlya* and *D. achlyoides* is definite. Although the exact nature of the active principle has not yet been determined, several of its properties are known. Its organic character is reasonably certain because of its destruction by complete ashing, although it is also relatively in-

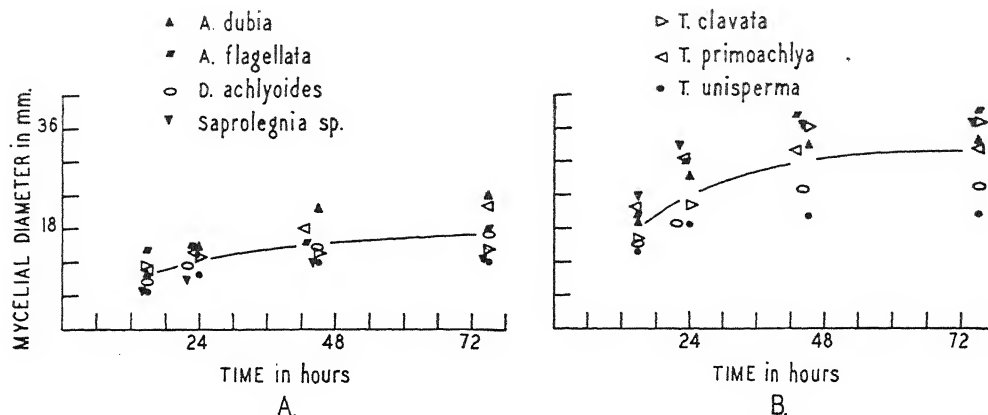


Fig. 5. Graph, showing the influence of the ether-insoluble fraction on the mycelial growth of seven different species of the Saprolegniaceae. (A, control; B, with ether-insoluble fraction.)

soluble in both ether and alcohol, relatively soluble in water, and resistant to prolonged heating. Its action does not become effective until a threshold concentration is attained, a process the rate of which is greatly hastened by increased oxygen tension. Furthermore, this active compound is not formed only by *T. primoachlya* and *D. achlyoides*, but is a component of the metabolic excretions of other fungi, such as *T. clavata*, *T. unisperma*, etc. In this connection, it is remarkable that its action can be imitated, although not equaled, by some ammonium salts.

The staling products of *T. primoachlya* also contain two compounds which control the vegetative growth not only of that species but also of many of the other Saprolegniaceae. One, soluble in ether and alcohol, greatly inhibits the rate and extent of mycelial development; the other, soluble in water but insoluble in ether and alcohol, markedly increases the rate and extent of vegetative growth. These two substances similarly must reach a threshold concentration before their properties become visibly effective.

The understanding of the mechanism causing the change in sporangia in *T. primoachlya* and *D. achlyoides* makes possible the establishment of the true taxonomic position of the two species. They are unusual in that under normal environmental conditions their genetic constitution can produce in large numbers two types of sporangia, each of which is characteristic of a separate genus. However, since the achlyoid sporangia are inhibited much more readily than those of the other type, the present classification is doubtless the correct one. Nevertheless, that such species are aberrant is obvious, although whether they are the primitive ancestors of the typical modern genera or the results of intergeneric matings is still a matter of speculation.

The fact that the asexual characters of different genera can be altered by physical or chemical components of the environment offers evidence of the very close phylogenetic relationship of the genera of the Saprolegniaceae. Since *Achlya flagellata*, which is characterized by the formation of achlyoid sporangia alone, as reported in the literature and verified by

the writer, can produce sporangia which dehiscence in a manner typical of the genus *Thraustotheca*, the protoplasm of that species must contain some latent characters that associate it with the genus *Thraustotheca*.

Further evidence for this close relationship of the genera appears in the following observations. (1) When *T. clavata* and *Dictyuchus* sp. were grown on hemp seeds in 50 cc. volumes of water at 95°F., many of the sporangia formed papillae of dehiscence at their apices as the spores within were being delimited (cf. Weston, 1918, p. 170). However, these papillae, resembling exactly those functioning in the sporangia of the species of either *Saprolegnia* or *Achlya*, were non-functional, for the sporangia dehiscence each in its normal manner, i.e., in *Thraustotheca*, by an irregular rupture of the external wall, and in *Dictyuchus*, by emergence of single zoospores from their individual cells. (2) When *Achlya* sp. was grown on hemp seeds in 50 cc. of a 0.001 per cent solution of the dye Bismark Brown, the spores in most of the sporangia were not discharged, but remained immotile and germinated *in situ* by the formation of either short vegetative hyphae or short hyphae at the end of which were borne tiny sporangia. This behavior is clearly that of the genus *Aplanes* as originally defined by de Bary (1888). (3) When a sporangium of *Saprolegnia* sp. developed in a hanging-drop of redistilled water at 10°C., the zoospores after emergence behaved in a manner typical not of that genus but of the genus *Protoachlya* (Coker, 1923), in that most of them were sluggish and formed an irregular clump at the sporangial mouth. (4) Under normal conditions, *T. primoachlya* and *T. unisperma* were observed to develop at rare intervals dictyuchoid sporangia, in which the spore initial walls fused with each other and with the outer sporangial wall, and in which the zoospores emerged individually.

In addition, Lechmere (1910, 1911), Collins (1920) and others have reported variations within a species growing in a normal environment which

further illustrate the tendency of the asexual characters of one genus to resemble those of another.

The foregoing observations indicate that under certain environmental conditions the asexual characters of one genus may change so as to resemble those of another, a situation which can take place only if the genera concerned are closely related phylogenetically. This is particularly true in a group such as the Saprolegniaceae, in which the genera are separated by their asexual characters. This close association is further corroborated by the very fact that species do exist which normally have two types of sporangia, each characteristic of a different genus.

SUMMARY

Three species of the Saprolegniaceae are recognized which have typically two methods of sporangium dehiscence, each of which is representative of a different genus: *Thraustotheca primoachlya*, *Diclyuchus achlyoides*, and *T. bispora*. Investigations on *T. primoachlya* and *D. achlyoides*, which produce achlyoid sporangia at first, indicate that some component of the staling material is capable of inhibiting the formation of the achlyoid sporangia. This active staling principle, which is not restricted to the foregoing two fungi but occurs in many others, must attain a certain threshold concentration before its

effect becomes evident. This principle, organic in nature, is probably not an ammonium compound, although ammonia is excreted in relatively large amounts and has some effects in common.

The mycelium of *T. primoachlya* showed responses to extracts of the staling material additional to the one of inhibition of achlyoid-sporangium-formation: (a) both an abnormally extensive vegetative growth and frequently a premature development of oogonia caused by the ether-insoluble fraction, and (b) inhibition of growth and of sporangium formation caused by the ether-soluble fraction. These effects were not restricted to the mycelia of *T. primoachlya* and *D. achlyoides*, but were apparent in the mycelia of other members of the Saprolegniaceae.

From the foregoing results the conclusions are drawn (1) that the successive production of sporangia of *T. primoachlya* and *D. achlyoides* is partly controlled by the environment; (2) that those species with two types of sporangial dehiscence are probably classified correctly; and (3) that the genera concerned are very closely related phylogenetically.

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FREEZABLE WATER CONTENT AND THE OXYGEN RESPIRATION IN WHEAT AND RYE GRAIN AT DIFFERENT STAGES OF RIPENING¹

Harold G. Shirk

SHIRK AND Appleman (1940) have shown that a close parallelism exists between the freezable water content and the oxygen respiration of mature wheat when the tissue colloids are in equilibrium with different amounts of imbibed total water. The purpose of the present investigation was to examine the type of relationship between the respiration and total natural water content and between the respiration and freezable water content in cereal grains during growth and ripening of the grain, using immature grain containing different amounts of total natural water.

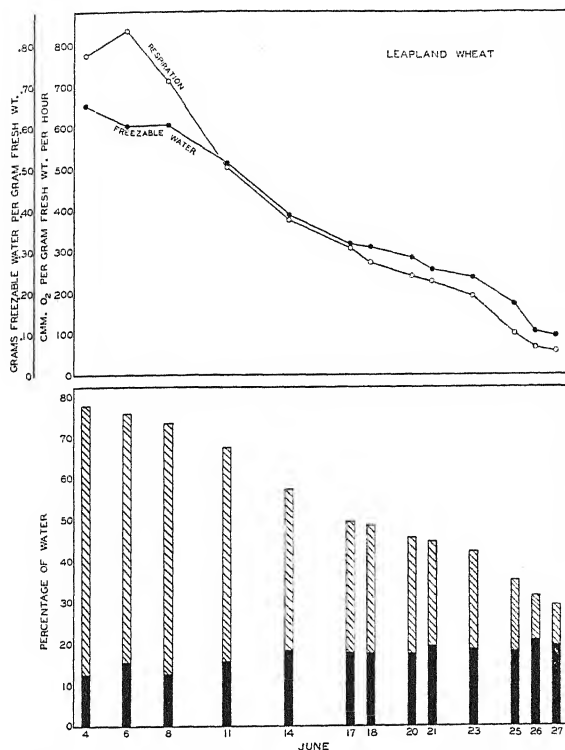


Fig. 1. The oxygen respiration and water relations of Leapland wheat at different stages of ripening calculated on the fresh weight basis. The height of the columns in the lower section indicates total water, the solid portions unfreezable water, and the shaded portions freezable water as percentage of the fresh weight.

EXPERIMENTAL PROCEDURE AND METHODS.—The wheat and rye grain samples were obtained from plots grown on the University Farm. On each sampling date 90 to 100 heads of grain were brought im-

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mediately to the laboratory. Duplicate respiration samples were taken by removing four kernels from the central portion of each head. Two kernels were used in each of the duplicate samples, making a total of 180 to 200 kernels in each respirometer. The time required for sampling varied from 75 minutes for the

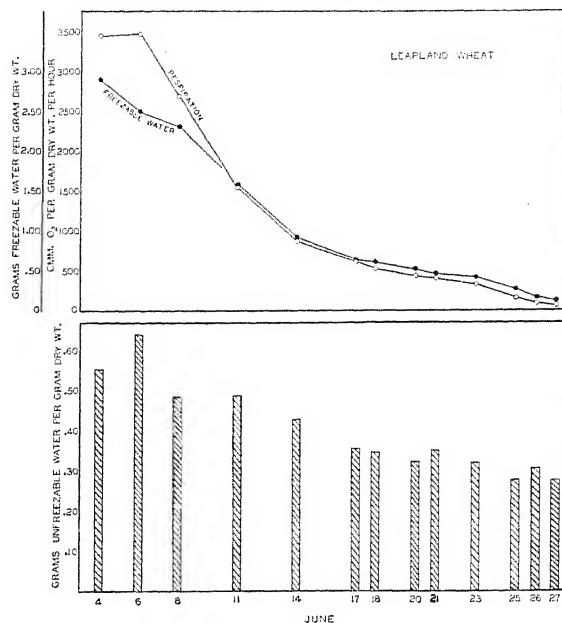


Fig. 2. The oxygen respiration and water relations of Leapland wheat at different stages of development calculated on the dry weight basis. The columns in the lower section indicate unfreezable water.

green milk stage grain to 15 minutes for the preparation of the more mature grain samples. After the respiration samples were weighed and placed in the respirometers, a second sampling was made of the same lot of heads removing four additional grains from the middle of each spike. After completing the duplicate respiration determinations, the grain in the respirometers was added to the second sampling of grain, mixed, and portions thereof were taken for triplicate freezable water determinations and duplicate total water determinations.

The total water determinations were made by drying at 80°C. in a vacuum oven under 3 to 4 cm. pressure. The total water content is expressed as percentage of the fresh weight.

The freezable and unfreezable water fractions were determined by the heat of fusion procedure described in detail by Greathouse (1935) with slight modifications as previously described by the author (1940). The freezable and unfreezable water data

TABLE 1. The oxygen respiration and water relations in Leapland wheat during growth and maturation. Freezable water samples were frozen at -25°C . (1940 study).

Date of sampling	Oxygen consumed per gram per hour		Freezable water		Unfreezable water		Ratio	
	Per cent total water	Fresh wt. Dry wt.	Per cent of fresh weight	Per cent of total water	Per cent of fresh weight	Per cent of total water	Freezable	Unfreezable
June 4	77.46	777	65.04	83.96	12.12	16.04	55.12	5.23
June 6	75.83	836	60.35	79.38	15.48	20.42	61.08	3.89
June 8	73.57	712	60.76	82.59	12.81	17.41	48.48	4.74
June 11	67.49	502	51.56	76.40	15.93	23.60	48.98	3.24
June 14	57.20	375	38.81	67.85	18.39	32.15	42.96	3.11
June 17	49.78	308	31.85	63.99	17.93	36.01	35.69	1.78
June 18	48.69	270	30.99	63.61	17.70	36.36	34.50	1.55
June 20	45.62	238	28.15	61.70	17.47	38.30	32.13	1.61
June 21	44.63	224	25.22	56.51	19.41	43.49	35.04	1.30
June 23	42.11	190	23.62	56.10	18.49	43.99	31.93	1.28
June 25	35.09	100	17.02	48.49	18.07	51.51	27.84	.94
June 26	31.50	64	10.56	33.51	20.94	66.49	30.57	.50
June 27	29.12	59	9.55	32.80	19.57	67.20	27.57	.49

are calculated on the fresh and dry weight bases as well as percentage of the total natural water.

The Warburg manometer and respirometer used in this investigation have been described in detail by Shirk and Appleman (1940). The samples were left in the respirometers at 28°C . for a 30-minute temperature equilibration period after which the respiration was measured for one hour. The results are expressed as cubic millimeters of oxygen at standard temperature and pressure taken up in one hour per gram fresh weight and per gram dry weight.

RESULTS.—*Wheat*.—Leapland² wheat grown in 1939 and 1940 was used for this portion of the investigation. The experiments with both wheat and rye from the 1940 crop were a repetition of those with the 1939 crop except for the freezing temperature. The results with the two crops were so similar that only the data from the 1940 study are presented in detail. The first sample of the 1940 wheat crop was collected about one week following pollination of the flowers at which time the total water content was 77.5 per cent of the fresh weight. Samples were taken at one-, two-, and three-day intervals until the total water content of the grain was about 29 per cent. The oxygen respiration intensity and the water relations of developing and ripening wheat are presented in table 1 and in figures 1 and 2.

In figure 1 the water fractions and the oxygen taken up by the grain in one hour are calculated on the fresh weight basis. Because of the difficulty sometimes encountered in interpreting free and bound water data calculated on the fresh weight basis when the total water content is a variable throughout the experimental period, the data were also calculated on the dry weight basis as shown in figure 2.

The respiratory activity is very high in the young grain. The peak of activity is reached eleven or twelve days after pollination followed by a rather sharp decline which becomes more gradual as the grain matures. The total water content (as indicated by the total heights of the columns in the lower section of figure 1) decreased rather gradually throughout the 1940 experimental period. The progressive decrease of the percentage of total water is paralleled very closely by the freezable water content, indicating that the loss of free water accounts for a large measure of the total water loss. There is a close parallelism between the freezable water content and the respiration intensity, calculated both on fresh and dry weight bases as shown in the upper sections of figures 1 and 2, except during the earlier stages of grain development. The histographic representation of the water fractions calculated as percentage of fresh weight in the lower section of figure 1 shows the gradual changing of the freezable-unfreezable water equilibrium shifting from a ratio of 5.23 (5.23 parts freezable water to 1 part of unfreezable water) in the first sample to a ratio of 0.50 for the last sample. Approximately 84 per cent of

²Leapland wheat is a natural selection of Leap wheat developed at the Maryland Agricultural Experiment Station.

TABLE 2. The oxygen respiration and water relations in rye grain during growth and maturation. Freezable water samples were frozen at -25°C . (1940 study).

Date of sampling	Per cent total water	Oxygen consumed per gram per hour		Freezable water			Unfreezable water			Ratio Freezable Unfreezable
		Fresh wt.	Dry wt.	Per cent of fresh weight	Per cent of total water	Per cent of total solids	Per cent of fresh weight	Per cent of total water	Per cent of total solids	
May 31	75.51	601	2,454	63.88	84.60	260.91	11.63	15.40	46.51	5.55
June 3	73.11	574	2,134	59.15	80.90	220.03	13.96	19.10	51.92	4.24
June 6	71.46	495	1,734	59.25	82.92	207.67	12.21	17.08	42.76	4.85
June 7	70.89	508	1,745	57.69	81.38	198.21	13.20	18.62	45.35	4.37
June 10	66.50	403	1,202	53.86	80.99	160.80	12.64	19.01	37.74	4.26
June 14	59.17	323	791	43.47	73.47	106.50	15.70	26.53	38.45	2.77
June 17	55.42	296	663	40.41	72.92	90.64	15.01	27.08	33.67	2.69
June 19	52.38	249	522	35.73	68.22	75.04	16.65	31.78	34.95	2.15
June 21	50.23	246	494	33.55	66.80	67.42	16.68	33.20	33.50	2.01
June 22	48.12	239	461	29.49	61.28	58.27	18.63	38.72	34.48	1.64
June 24	44.52	188	338	26.68	59.92	48.09	17.84	40.08	32.16	1.50
June 25	42.75	183	267	22.77	53.97	39.78	19.98	46.73	34.89	1.14
June 28	31.68	82	120	11.71	36.96	17.14	19.97	63.04	29.22	.59

the total water present in the first sample of wheat grain was free water or freezable at -25°C . As development and ripening took place a smaller percentage of the total water was freezable, until finally when the grain had 29 per cent total water only approximately one-third of it was freezable.

The tendency for one gram dry matter to associate less unfreezable water as ripening of wheat grain takes place is rather interesting, indicating either

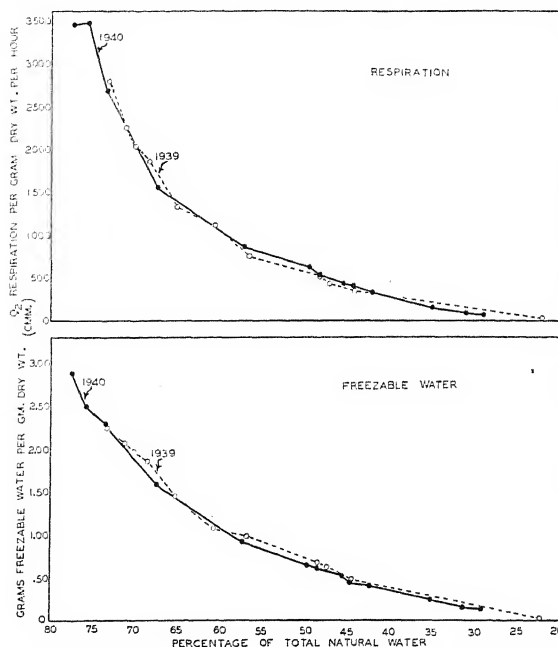


Fig. 3. The oxygen respiration and freezable water of Leapland wheat grain at different natural total water contents. The 1939 freezable water samples were frozen at -15°C ; the 1940 samples were frozen at -25°C .

an increase of hydrophilic colloids or a higher water-binding capacity of the colloids present in the grain immediately following their formation.

In figure 3 are presented the respiration and freezable water data from both the 1939 and 1940 wheat studies. The respiration intensity and the freezable water per gram dry weight are plotted against the total water content. In the 1939 study the freezable water samples were frozen at -15°C , instead of -25°C , as in the 1940 study. The conditions for the respiration and total water measurements were otherwise the same for the two years. The respiration results are surprisingly similar for the two years' work. A comparison of the freezable water curves for the two years shows that they coincide very closely, indicating that no more water was frozen from the 1940 wheat grain when frozen at -25°C , than from similar grain grown in 1939 and frozen at -15°C . The biocolloidal system of wheat at the different stages of ripening and development included in this investigation is evidently in equilibrium with a given amount of unfreezable water at -15°C , which

cannot be frozen by lowering the temperature to -25°C .

The parallelism between the fall of the respiration intensity and the decrease of freezable water as wheat grain ripens is again easily recognized from the curves in figure 3. The curves formed by plotting the respiration and freezable water against total water of the immature grain resemble logarithmic type, similar to the curves formed by plotting respi-

study during the same early phase of kernel development.

A comparison of the oxygen respiration intensity in tables 1 and 2 for the wheat and rye at approxi-

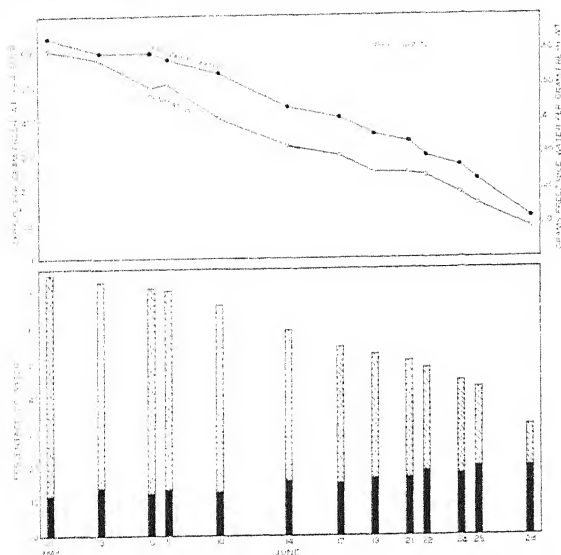


Fig. 4. The oxygen respiration and water relations of rye grain at different stages of development calculated on the fresh weight basis. The height of the columns in the lower section indicates total water, the solid portions unfreezable water, and the shaded portions freezable water.

ration and freezable water against the imbibed total water of mature wheat grain (1940).

Rye.—The Abbruzzi rye used in this experiment was collected at one-, two-, and three-day intervals during the ripening and maturation of the grain. The first sampling during the 1940 study was taken about one week following pollination, at which time the total water content was 75.5 per cent of the fresh weight. The water relations and oxygen respiration of rye grain at different stages of development and ripening during the 1940 season are presented in table 2 and shown graphically in figures 4 and 5.

From the upper sections in figures 4 and 5 it is apparent that there is a close parallelism between the declining rate of oxygen respiration and the decreasing amount of freezable water as rye grain develops and ripens. This parallelism is equally apparent whether calculated on the dry weight or on the fresh weight basis. The time rate of decline for both the total water and freezable water was more gradual and less rapid for the 1940 crop of rye during the earlier stages of development than for the 1940 crop of wheat during the same period. Samplings during the 1939 rye study were not started early enough to make a similar comparison with the 1939 wheat

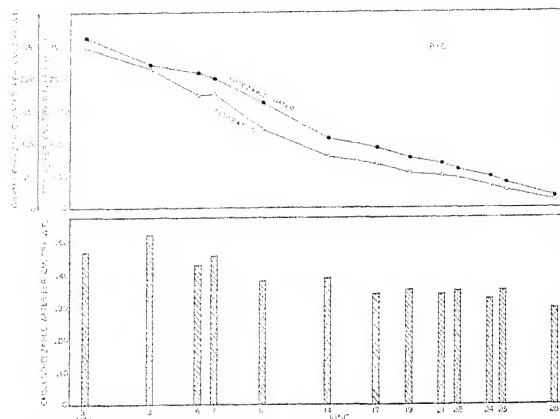


Fig. 5. The oxygen respiration and water relations of rye grain at different stages of development calculated on the dry weight basis.

mately equal stages of development shows the oxidative processes of the wheat grain to be somewhat higher than in rye. The tendency for one gram of dry matter to associate less unfreezable water as the rye ripens is shown in the lower section of figure 5, with the drift of this tendency not as pronounced as with the wheat. The freezable-unfreezable water equilibrium as presented in the lower section of figure 4 shifted from a ratio of 5.5 parts freezable to 1 part unfreezable water for the first sampling to 0.60 for the last sampling.

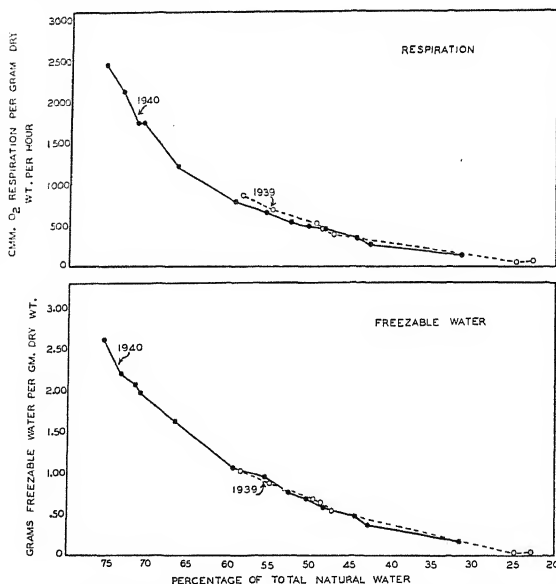


Fig. 6. The oxygen respiration and freezable water of Abbruzzi rye grain at different natural total water contents. The 1939 freezable water samples were frozen at -15°C ; the 1940 samples were frozen at -25°C .

In figure 6 are presented the results for the 1939 and 1940 rye studies. The data in this graph are plotted against the total water content instead of time as in figures 4 and 5. The respiration and total water were determined by the same procedures in the two studies, but the freezable water samples in the 1939 study were frozen at $-15^{\circ}\text{C}.$, and the 1940 samples at $-25^{\circ}\text{C}.$

From the freezable water data plotted in the lower section of figure 6 it is evident that no more water was frozen at $-25^{\circ}\text{C}.$ in the 1940 rye than at $-15^{\circ}\text{C}.$ in the 1939 rye.

The closely duplicated curves formed by plotting the 1939 and 1940 respiration rates against the total water are closely paralleled by the similarly duplicated freezable water curves as plotted in the lower section of figure 6.

SUMMARY

The Warburg manometer was used to measure the oxygen respiration of rye and wheat grain at different stages of ripening in relation to the freezable water content and total natural water. The freezable water was measured by the heat of fusion technique; the 1939 rye and wheat samples were frozen at $-15^{\circ}\text{C}.$ and the 1940 samples at $-25^{\circ}\text{C}.$

Comparing the average results from the two years' work with immature rye and wheat, there was no apparent difference between the amounts of water freezable from the respective 1939 grain samples when frozen at $-15^{\circ}\text{C}.$ and the 1940 samples frozen at $-25^{\circ}\text{C}.$

As the young green grain of rye and wheat developed and ripened, the free water in the grain decreased. At the same time, there was a similar time rate decrease in the amount of oxygen taken up in one hour per gram fresh weight or per gram dry weight of grain which paralleled the time rate decrease of the freezable water in each case. This same close relationship was shown when the respiration data and freezable water results were plotted against the total water instead of time. The descending and paralleling respiration and freezable water curves for immature rye and wheat when plotted against time or against total water were very closely duplicated in the 1939 and 1940 studies.

Both wheat and rye showed a tendency to associate less bound water per gram of dry grain as development and ripening took place. This tendency which was most pronounced in ripening wheat grain was however relatively small when compared with the loss of free water during the same time; so that there resulted a large shifting of the free-bound water equilibrium.

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THE PHOTODYNAMIC ACTION OF NEUTRAL RED ON ROOT TIPS OF BARLEY SEEDLINGS. PART II. ABNORMALITIES OF CELLS AND TISSUE¹

Elizabeth Knight Patterson

A CYTOLOGICAL study was made of the photodynamic action of neutral red on the root tips of seedlings of *Hordeum vulgare*. A previous paper, Part I (Patterson, 1941), describes the methods used and the resulting fall in the frequency of cell division. Three groups of roots were used: Group E, the experimental roots, which were immersed in a 1:75,000 solution of neutral red and irradiated with sunlight for one hour; Group NRC, the neutral red controls, which were kept in the dye solution in the dark;

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Parts I and II comprise a dissertation presented to the faculty of Bryn Mawr College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The subject of this investigation was suggested by the late Dr. David H. Tennent and the author is glad to take this opportunity to express her appreciation for his valuable advice and criticism during the course of the work. She is also grateful to Dr. William L. Doyle and Dr. Walter C. Michels for their invaluable assistance in the preparation of the photomicrographs. The author wishes to thank Dr. Mary S. Gardiner and the Department of Biology of Bryn Mawr College for the laboratory facilities placed at her disposal.

Group SC, the sun controls, which were put in distilled water and irradiated with direct sunlight simultaneously with Group E. In one experiment another group was added, Group DC, the dark controls, which were kept in distilled water in the dark. All groups were grown in the dark, and the dye solution in Groups E and NRC was replaced with distilled water one hour after irradiation.

Counts of mitoses in sections of the experimental roots fixed at various intervals after irradiation (0-70 hours) showed that there was a sharp decrease in the frequency of cell division, the roots becoming mitosis-free about seven hours after irradiation. The neutral red controls maintained a high frequency of cell division, whereas the sun controls showed a 40 per cent decrease in the number of divisions present. After twenty hours sections of both sun and neutral red controls had normal numbers of cell divisions. No abnormalities of nuclear division were seen in the control roots. The roots irradiated in neutral red solution and fixed 20 to 70 hours after irradiation ex-

hibited three types of response to the photodynamic injury: (1) division started again, (2) the roots continued to mature, and (3) the cells died progressively inward toward the center of the root.

A study of the division cycle in the experimental roots suggested that, after an initial decline in the number of cells entering division, there was a group of cells retarded in interphase which went through

greater in the cells on one side of the root than on the other. In material fixed two hours after the end of irradiation a continuation of this reaction was seen. The small vacuoles coalesced, and four hours after irradiation the lower meristem cells contained only a few large vacuoles (fig. 2). These vacuoles were filled with a precipitate in some cases and in others with a homogeneous mass of unstained mate-

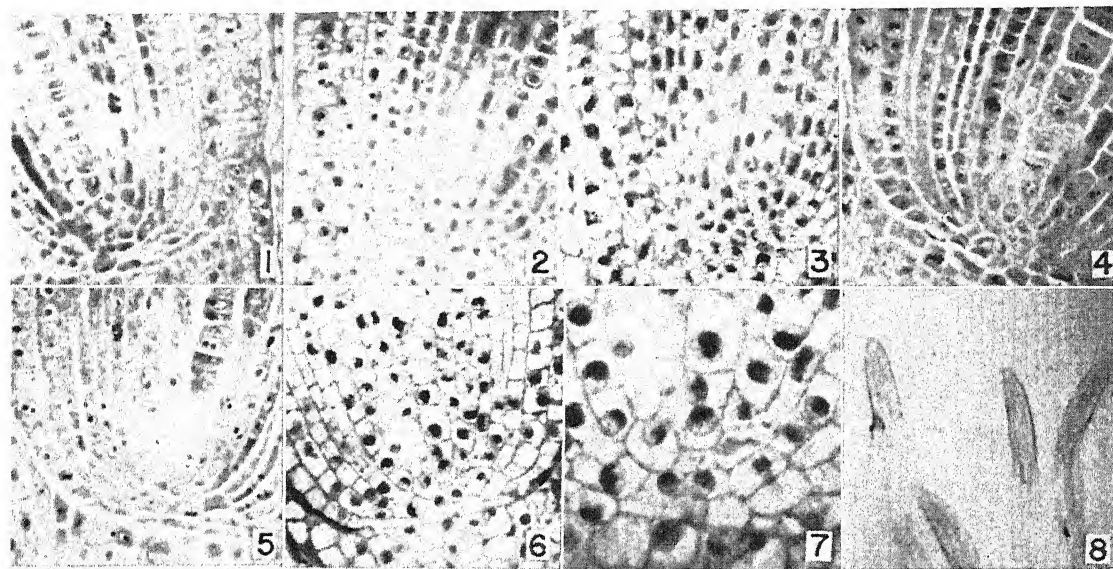


Fig. 1-8. The progress of vacuolization in barley roots irradiated with sunlight in a 1:75,000 solution of neutral red and fixed at various intervals after irradiation. Photomicrographs were taken with an Argus camera using 35 mm. Panatomic-X film and enlarging. Longitudinal 6-micron paraffin sections were stained by Heidenhain's iron haematoxylin procedure with the exception of the several Feulgen preparation as noted. All roots were taken from the experimental group and from experiment I unless otherwise noted.—Fig. 1. Median longitudinal section of tip at junction with root cap; one hour after irradiation; note small vacuoles appearing in cells of the dermatogen. $\times 260$.—Fig. 2. Four hours after irradiation; vacuoles becoming larger and appearing in cells of the periblem. $\times 260$.—Fig. 3. Nine hours after irradiation; vacuoles now large and present in periblem and plerome; no dividing cells. $\times 260$.—Fig. 4. Section of root, control in neutral red, fixed simultaneously with root in figure 3. Cytoplasm dense, vacuoles very small; cell division normal in form and occurrence. $\times 260$.—Fig. 5. Section of root, sun control, fixed nine hours after irradiation with sunlight in distilled water; cytoplasm dense, no vacuoles visible; cell division normal in form, somewhat diminished in occurrence. $\times 260$.—Fig. 6. Twenty-three hours after irradiation; cells even in plerome contain a large vacuole. General appearance of root tip very similar to region of elongation of a normal root. $\times 260$.—Fig. 7. Central region of figure 6 in higher magnification. Cells resemble maturing cells; walls thickened, cytoplasm in a narrow strip close to cell walls; chromatin lies in an abnormally heavily staining net. $\times 560$.—Fig. 8. Section of four roots fixed twenty-three hours after irradiation; figures 6 and 7 give the tip of one root in higher magnification. Dark lines on edge of roots caused by dead cells staining intensely with haematoxylin. Two more or less straight roots have approximately equal numbers of dead cells on both sides of root; two bent roots have more dead cells on concave side. $\times 16$.

division, after which a gradual falling off took place until no new cells started division. In one experiment an arrest in metaphase also occurred but the injury to these cells was so great that death resulted.

VACUOLIZATION AND MATURING OF CELLS AND TISSUE.—Sections of barley roots that had been irradiated in neutral red and fixed immediately after the end of irradiation could not be distinguished from the controls as far as their general appearance was concerned. The cytoplasm in the lower meristem cells was dense, and there were no visible vacuoles even in the dermatogen. In the tips of roots fixed one hour after irradiation the cells in the dermatogen showed many small vacuoles (fig. 1). Their occurrence was

rial. This may or may not have been neutral red. The vacuolization was so extensive in the nine-hour material that the sections appeared lacy (fig. 3). The cytoplasm of the meristem cells of all the controls remained dense throughout the experiments. Figure 4 from the nine-hour neutral red control and figure 5 from the nine-hour sun control serve as examples of the whole series of controls.

A study of sections of material (Group E) fixed twenty-three hours after irradiation in dye solution (fig. 6-7) showed that all of the cells of the dermatogen and periblem and many of the cells of the plerome contained one large vacuole. The cell walls were thickened, the cytoplasm limited to a narrow

band close to the walls and surrounding the nuclei. The nuclei had assumed the small and compact appearance of those normally found in the zone of elongation of the root. Such cells will be referred to below as "maturing." In the irradiated roots acid fixation was less apt to produce a vacuole in the nucleus than in similar cells in normal roots. The chromatin lay in an abnormally heavily staining net often in an eccentric position in the nucleus. The net masked the nucleoli which were small, again like those in the zone of elongation. There was no sign of any cell division in this twenty-three-hour material and the meristematic character of the root was, therefore, entirely lost. The whole picture suggested that, following arrest of division, the cells had matured. The main difference between these cells and those normally found in the zone of elongation was that the longitudinal dimensions of the latter were usually greater than their transverse measurements. The cells in the tip in the twenty-three-hour material were approximately equal in length and breadth. The mature character of the central row of cells was striking. At this stage the tracheids with spiral thickenings did not extend farther toward the tip in the irradiated than in the control roots.

Pekarek (1927) described in detail a similar premature aging of the root tissue of *Vicia faba* after treatment with X-rays. He states that one day after irradiation the cells had the same appearance as they would have had in the normal root in the region approximately two millimeters behind the initial zone. A study of the appearance and measurement of the size of the resting nuclei added data to his observations on maturation due to vacuolization of the roots. Pekarek's work confirmed that of Koernicke (1905) on the effect of X-irradiation on growth of roots. Koernicke pointed out that this premature aging of the meristem is a typical phenomenon in young plants that are inhibited in growth. The non-specific nature of the effect was also evident from the present experiments in which injured roots of both neutral red and sun control groups showed maturing cells at the tip.

When the vacuolization reaction was very severe, death of the cells followed. This was the case with the stronger intensities of sunlight, and with the most sensitive root tips. In experiment I, material fixed seven hours after irradiation in dye solution showed many dead cells in the dermatogen, more on one side of the root than the other. Greater injury occurred on the side of the root toward the sun. From this time on, every group of experimental roots fixed showed varying numbers of dead cells in the sections of one or more of the roots. Sections of four roots fixed twenty-three hours after irradiation are shown in figure 8. The dark lines on the edge of the sections were caused by dead cells that had stained intensely with haematoxylin. The roots that were approximately straight had about equal numbers of dead cells on both sides; the bent roots had more dead cells on the concave side. This bending will be discussed in detail later.

Many sections were found with three or more rows of dead cells on the periphery. This was especially characteristic of the material in experiments II, III, and IV (see Patterson, 1941, tables 1 and 3). Here dead cells appeared in the roots fixed as early as two hours after irradiation in dye solution. A section of such a root in which part of the dermatogen is torn off is given in figure 9. The normal condition of the neutral red control for this time is shown in figure 10. In the irradiated roots fixed a day later, death of the cells had progressed inward to such a degree that only the cells of theplerome remained alive. The cytoplasm in these cells exhibited that peculiar granular appearance often indicating a stage in the process of degeneration. The chromatin in the dividing cells (all abnormal) stained intensely with haematoxylin, that in the resting cells very faintly. Roots in this condition did not recover, and the cell divisions arrested originally could be seen until the root completely degenerated. This severe reaction was particularly characteristic of the material in experiment IV.

Division reappeared in thirteen (24 per cent) of the fifty-five roots examined twenty or more hours after irradiation in neutral red. The mitotic figures were normal in every respect (fig. 13, 14). In the cells that first started to divide after the mitosis-free interval, the group of chromosomes was often pushed to one side of the cell, or compressed in the center of the cell due to the presence of large vacuoles (fig. 14). This was especially true of divisions in the periblem. Such roots when allowed to grow for a period of twenty-two or more hours before sectioning were found to have root tips of normal character in which the cytoplasm of the cells was dense. In many cases divisions reappeared after a bending of the root occurred. Dead cells were always seen on the inner side of the bend (fig. 12, 15, and 19). It seems justifiable to assume that the root had been injured and that if division had not been completely arrested, it at least had been curtailed. In figure 13 new divisions are shown on the right of the central row of cells. These latter cells showed signs of injury, being stained intensely with haematoxylin (fig. 12). The divisions were, therefore, on the convex side of the root.

Further evidence of a previous injury to the older roots that showed division figures is given in figure 11. The row of dead cells on the periphery of the root was being sloughed off and the outer row of cells of the periblem was taking their place. These cells now had the columnar shape of cells of the dermatogen, and the new mucilaginous coat that they secreted may be seen (arrow) as a grey line between them and the black dead cells.

Koernicke (1905) in describing the effect of Ra irradiation on the growth of roots of *Vicia faba* seedlings noted that the cells of the root cap, as well as the original cells of the dermatogen, died and were sloughed off as the root resumed normal growth. Cells from the periblem then formed the outer layer of root cells and root hair formation did not occur.

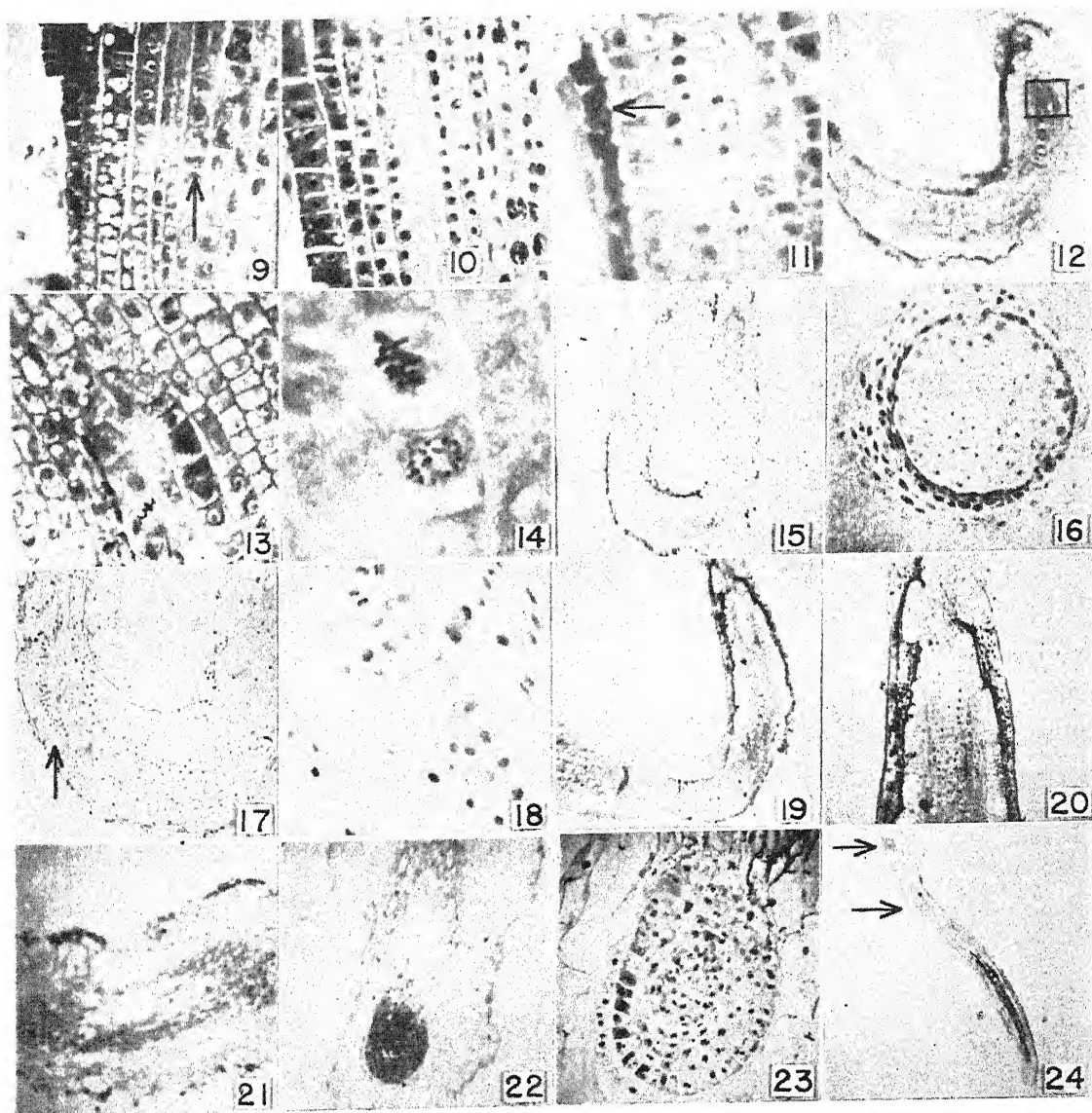


Fig. 9-24. Death and bending of roots and formation of branch roots.—Fig. 9. Edge of section of root tip fixed two hours after irradiation in experiment IV. Dermatogen cells dead, some sloughed off, and outer rows of periblem cells dead or dying; granular appearance of cytoplasm of inner periblem cells abnormal. Arrow points to a pseudoamitosis in which cytoplasmic bridge on one side is causing a deviation of upper daughter nucleus. $\times 260$.—Fig. 10. Comparable area in section of corresponding neutral red control root; vacuoles smaller and cytoplasm more homogeneous than in irradiated root; divisions normal; no dead cells. $\times 260$.—Fig. 11. Edge of section of root fixed $44\frac{1}{2}$ hours after irradiation in experiment II; normal division resumed; row of dead cells (black) on periphery sloughing off along with its heavy mucilaginous outer layer (grey). Outer row of periblem cells taking their place now have columnar shape of dermatogen cells. Arrow points to grey line of new mucilaginous layer secreted by these cells. $\times 260$.—Fig. 12. Section of root fixed twenty-five hours after irradiation in experiment III; number of dead cells lining concave side of bend greater in region of elongation than at tip. Few dead cells on convex side where tension caused rupture of outer cell row. Periblem cells on convex side swollen; plerome displaced toward concave side at tip; dead cells in central row. $\times 60$.—Fig. 13. Region in figure 12 enclosed in square given in higher magnification. Normal divisions on convex side of root; cell walls thickened and large vacuoles still present in many cells. $\times 260$.—Fig. 14. Another section of root given in figures 12 and 13. Chromosomes in prophase and in normal metaphase plate are pushed to side of cell by vacuoles. $\times 1,000$.—Fig. 15. Section of root fixed twenty-eight hours after irradiation in experiment IV; root bent into loop; tip growing downward roughly perpendicular to plane of figure. More dead cells on concave than on convex side of bend, periblem cells swollen both sides. $\times 27$.—Fig. 16. Cross section in higher magnification of tip of root in figure 15; dead cells outline root; some cells of cap dead. Many divisions present especially in plerome which lies in an eccentric position toward side of root with greater number of dead cells. $\times 116$.—Fig. 17. Another section of root in figures 15 and 16; Feulgen preparation; periblem cells on concave side of root swollen. Arrow points to branch root

PHOTOTROPIC RESPONSE.—Roots of barley seedlings that had been irradiated in sunlight for one hour in a 1:75,000 solution of neutral red, and subsequently kept in the dark for twenty hours or more, exhibited a marked ageotropic bending. During the first hour of the period in the dark the roots remained in the neutral red solution, which was replaced by distilled water for the remainder of the period. The roots bent sharply upward in the direction from which the light had come. In some roots growth was permanently arrested at this stage, in others division began again and the root grew downward. This resulted in the formation of loops or U's. Out of eighty-three controls kept in the dark in a solution of neutral red of the same concentration, only one showed any signs of bending. Root tips irradiated in sunlight for one hour in distilled water behaved normally. The experimental arrangement was such that geotropic bending was hindered since the roots were growing in water only 4–5 mm. in depth.

In a growth experiment roots were irradiated in neutral red solution and left for seventy hours after irradiation. Observation of the whole roots at the end of this period showed that out of forty-four, eighteen or 41 per cent exhibited ageotropic bending, while the control roots were all straight. Six of the bent roots continued to grow, but growth was arrested in the remaining twelve (see table 6, Part I, Patterson, 1941).

The data from roots fixed in the interval 20 to 70 hours after irradiation (table 3, Part I) showed that in fifty-five irradiated roots, twenty-three or 42 per cent bent upward. Sections revealed that cell divisions were present in only eleven of these. Only those roots that had more dead cells on one side than on the other were bent. This is evident in figure 8, which shows four roots in which division was arrested. The tip of one of these roots, given in higher magnification in figures 6 and 7, shows the mature nature of the cells. The section in figure 19 is of a root in which a few cell divisions had started. The tip of this root, shown in figure 20 in a higher magnification, reveals an odd pattern of the dead cells. The cells of the periblem in the row next to the dead cells were exceedingly swollen, especially in the transverse direction. Even when there were dead cells in the plerome, divisions were present in adjacent cells. Another section of a bent root (fig. 12) shows the pattern of dead cells characteristic of the majority of roots showing bending. The dead cells extended along the concave

side of the root, the number of rows being greater in the region of elongation than at the tip. A few dead cells were seen on the convex side. The tension here must have been so great that the outer row of cells was ruptured. This was found in all the bent roots. Comparison of cells of the periblem of the root represented in figure 17 with those of the root shown in figure 12 demonstrated that in the former they were more swollen on the concave side of the root, in the latter on the convex side. Another section of the same root as that used in making the Feulgen preparation of figure 17 is shown after staining with haematoxylin in figure 15. In every root examined, however, dead cells occurred on the concave side of the bend. It, therefore, seems reasonable to assume that the bending of the roots was caused either by a contraction, or a lack of elongation of one side of the root due to the dead cells. That side must have faced the sun so that the cells were killed by the photodynamic action of the neutral red.

As mentioned before, new cell divisions began in the central cylinder on the convex side of the root. This served to increase the curvature of the root and eventually produced a loop (fig. 15). The tip of this root is bent downward in a direction roughly perpendicular to the plane of the figure. This was, therefore, cut in cross section and is shown in figure 16 in higher magnification. Dead cells could be seen outlining the root, and some of the cells of the root cap were also dead. Many divisions were seen, especially in the plerome which was in an eccentric position, lying toward the side of the root with the greater number of dead cells. This shift of position may have been caused by a contraction of the dying cells on one side of the root while a simultaneous elongation of the cells on the opposite side was taking place. Again the frequency of division may also have been greater on the convex side. In all the roots containing more dead cells on one side, the plerome was displaced toward that side at the tip of the root due to contraction of the dead cells (fig. 12).

Metzner (1923) found that a positive phototropism could be induced in the roots of wheat seedlings by growing them in non-toxic dilute solutions of erythrosin (1:20,000–1:50,000) and rose bengal (1:500,000). In distilled water the roots exhibited only their normal geotropic bending when exposed to light. He distinguished two degrees of response which occurred, depending on the intensity of the light and the concentration of the dye used. In "pas-

forming on convex side of main root. $\times 50$.—Fig. 18. Branch root in figure 17 shown in higher magnification; giant nucleus belongs to cell in a vessel. $\times 260$.—Fig. 19. Section of a root fixed twenty-five hours after irradiation in experiment III; root bent upward and growing. Note odd pattern of dead cells. $\times 27$.—Fig. 20. Tip of root given in figure 19 given in higher magnification. Dead cells present on both sides of root, extending into plerome in places; periblem cells adjacent to dead cells exceedingly swollen in transverse direction. $\times 60$.—Fig. 21. Section of bend in root fixed 44½ hours after irradiation in experiment II. Root tip grown away from this point; dead cells on concave side bend and two branch roots starting to form. $\times 60$.—Fig. 22. Section of bend in a root fixed seventy hours after irradiation in experiment II; main root tip grown about 30 mm. from this point; branch root developing in connection with pericycle opposite xylem strand. $\times 150$.—Fig. 23. Branch root in figure 22 in higher magnification; organization of cells into plerome and periblem; endodermis of main root pushed ahead of branch root and about to rupture. $\times 260$.—Fig. 24. Forty-four hours after irradiation in experiment III; arrows point to two branch roots developing 2.1 and 2.8 mm. from apex of main root. Many dead cells in plerome of main root; no divisions present. $\times 16$.

TABLE I. *Abnormalities of cell division in roots irradiated for*

Hours after irradiation	Metaphase				Total	Anaphase		
	Total	Pycnotic	Scattered	Per cent abnormal		Number of cells With bridges	With deviation ^c	Per cent abnormal
0	150	4 d ^b	0	3	77	14 (7d)	(1)	18
1	88	20	19	44	52	26	(10)	50
2 I	57	31	5	63	26	19	(11)	73
2 II	14	0	1	7	6	0	(0)	0
2 IV	84	9	68	92	3	3	(0)	100
3	12	6	1	58	2	2	(2)	100
4	5	2	3	100	0	0	(0)	..
5	2	1	1	100	6	6	(2)	100
7	0	0	0	..	0	0
9	0	0	0	..	0	0
23	0	0	0	..	0	0
28 IV	66	19	45	97	4	4	(0)	100
34½ IV	42	28	13	98	3	3	(0)	100

^a Data from experiment I unless noted otherwise in the first column.

^b d denotes cells of the dermatogen.

^c Included in count of cells with bridges.

sive Krümmung" the roots bent very sharply toward the light (50 "K" frosted metal filament lamp at 25 cm., 1:50,000 erythrosin), while in "active Krümmung" (lamp at 40 cm., 1:500,000 erythrosin) the bending was more gradual. Metzner attributed the response in the case of "passive Krümmung" to an irreversible injury to the epidermal cells on the side of the root toward the light. He could see macroscopically that the root soon became stained red on one side, only dead cells taking up the acid dye. These dead cells could not elongate as did the underlying uninjured cells to which they were firmly connected. Metzner believed that the resulting bending was, therefore, purely mechanical in origin, hence "passive Krümmung." The bending continued until the roots were growing directly toward the light. Metzner explained that, since the root was then shaded by the root cap, the meristem cells were no longer injured and the root continued to grow. He also mentions that the tension on the shaded side of the root was often great enough to cause tearing of the epidermal cells. This was frequently seen in sections of bent roots in the present work.

In Metzner's "active Krümmung," the bending is less sharp and again the growth of the irradiated side falls behind that of the shaded side. But here Metzner believes that the oxidation in the cells facing the light merely influences the velocity of their growth. The bending started in the zone of elongation and, according to Metzner, corresponded exactly to that seen in other tropic reactions. An anatomical study showed that the cells on the concave side were still fully turgid, and Metzner could not recognize any visible staining of these cells. Microtome sections showed no injury to nuclei, and normal cell divisions were seen in the pericycle of the

bending zone. Root hairs were sometimes shorter on the irradiated than on the shaded side of the root.

The results from the present cytological study (using a basic dye that freely entered the cells) agreed completely with Metzner's findings (with an acid dye) in the case of his "passive Krümmung." However, all the roots exhibited a greater degree of injury than that shown in "active Krümmung." Possibly with more dilute dye solutions and less intense light, the latter reaction could be obtained with neutral red. Roots were found that exhibited only slight bending, but in them growth was arrested and the cells were maturing.

Blum and Scott (1933), after repeating Metzner's experiments, concluded "that Metzner's types simply represent different degrees of the same process" (p. 527). They were of the opinion that the phototropic orientation was accomplished by a greater elongation of the cells on the shaded side than on the illuminated side of the zone of active growth. The possibility was also mentioned that with roots in dye solutions, the photochemical reaction may only serve to alter the permeability of the growing cells to the acid dye, the side of the root taking up more dye being delayed in growth. The bending of the roots observed in the present experiments was very similar to that shown in the figures in the paper by Blum and Scott.

Blum and Scott found that wheat roots in dye solution in the dark exhibited delayed growth and unoriented bending similar to that described by Boas and Merckenschlager (1925). These latter investigators found that roots from barley grains, germinated in the dark in Petri dishes containing solutions of eosin (1:3,000 to 1:640,000), grew in all directions, some directly upward. This ageotropic growth was not exhibited by grains germinated in neutral

one hour in sunlight in a 1:75,000 solution of neutral red.^a

one hour in sunlight in a 1:75,000 solution of neutral red.

Total	Telophase			Total	Resting cells				Number of binucleate cells
	Number of cells				Number of pseudoamitoses				
	With chromosomal bridges	Plate abnormal	Per cent abnormal		Chromatin in stalk	Cytoplasm in stalk	Bilateral retraction	Unilateral retraction	
89	3	0	3	1	0	0	0	0	7 (4d)
39	5	6	28	15	3	9	2	1	23 (11d)
31	3	6	29	5	0	3	1	1	24
7	0	0	0	5	0	5	0	0	6 d
6	3	2	83	19	9	9	1	0	11
4	0	0	0	5	1	4	0	0	22
1	0	0	0	0	0	0	0	0	13
0	0	0	..	2	0	2	0	0	12
0	0	0	..	14	0	13	1	0	13
0	0	0	..	2	0	0	2	0	4
0	0	0	..	2	0	2	0	0	4
14	12	0	86	18	9	4	0	5	12
1	0	0	0	14	12	2	0	0	10

red (1:3,000 to 1:12,000). These experiments were verified and extended by a number of other investigators whose work has been reviewed by Boysen-Jensen (1934). Boysen-Jensen found that roots exhibiting this type of ageotropic behavior contained no growth substance in their tips.

In the present experiments, only one case of bending was seen in roots grown in the dark after two hours' immersion in neutral red (1:75,000). The injury to the irradiated roots was so great that the bending could only be compared with Metzner's "passive Krümmung," due to unilateral death of cells. No determination of the presence of growth substances was made.

FORMATION OF BRANCH ROOTS.—Study of sections of material fixed 20 to 70 hours after irradiation in a solution of neutral red revealed that one to four branch roots were formed in eight out of the thirteen roots in which new cell divisions were visible (Patterson, 1941, table 3). These branch roots usually developed in the bend of the main root and extended toward its convex side, *i.e.*, away from the dead cells. The arrow points to such a root in the Feulgen preparation shown in figure 17. This branch root is shown in higher magnification in figure 18. The large nucleus to the right of the branch root was a giant nucleus of a cell in a vessel. Two branch roots were starting to form in the bend of the root shown in the section in figure 21. That these groups of cells actually represented embryonic branch roots is illustrated by a section of a root fixed seventy hours after irradiation (fig. 22). The root can be seen emerging from the stele opposite a xylem strand. Organization of the cells into plerome and periblem regions is shown in figure 23 which is a higher magnification of figure 22. The endodermis of the main root was being pushed out ahead of the branch root, and it was about to rupture. Every group of meristematic tissue seen in the mature region of the roots occurred in connection with the pericycle, and even the smallest groups

showed the organization characteristic of branch root formation (see Patterson, 1941, fig. 2).

Some relatively straight roots were found in which branch roots had developed close to the tip. Such a root is shown in figure 24. Two branch roots were developing 2.1 and 2.8 mm. from the apex of the main root. The meristematic cells of the latter showed no division, and there were many dead cells in the plerome. This root was similar in appearance to the onion roots described as occurring after hormone treatment by Levan (1939). These roots after growing four to six days in dilutions (1,000 to .001 ppm.) of indole acetic acid showed a decrease in the number of divisions in the apical meristem until none were seen. The tip often atrophied. Simultaneously many dividing cells appeared in the exterior layers (pericycle) of the stele. Sometimes these cells were uniformly dispersed, but usually they were grouped and gave rise to lateral roots. Levan also described dividing cortical cells, the chromosome complement of which had often been doubled or quadrupled. No such cells were seen in the irradiated barley roots. Swellings above the meristem due to the sudden enlarging of the cortical cells as described by Levan after hormone treatment were seen in the present experiments in occasional control and experimental roots that were well supplied with root hairs.

A control root (sun) fixed 34½ hours after irradiation showed formation of two branch roots. The main root tip was injured, showing many dead cells. Sections of a control root (neutral red) fixed 44 hours after irradiation showed an abortive attempt at the formation of a branch root. The group of meristematic cells were all dead. The main root was bent and there was no division visible in the tip, the cells being mature. All control roots with normal meristematic tissue in their tips showed no sign of formation of branch roots.

In order to find out if branch roots appeared normally under the conditions of these experiments,

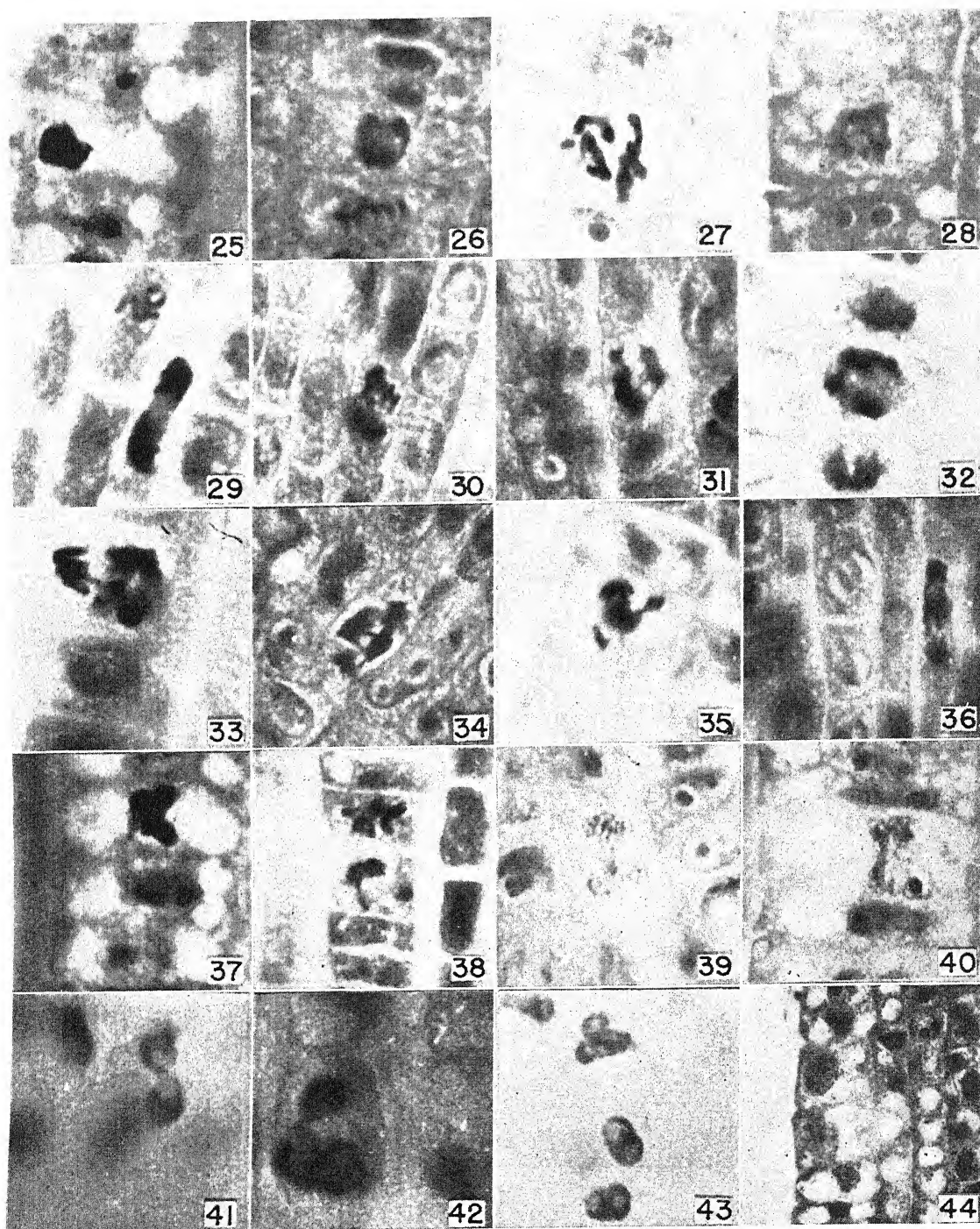


Fig. 25-44. Abnormalities in the division figure.—Fig. 25. Immediately after irradiation; pycnotic metaphase in dermatogen cell which lies about .5 mm. from the tip; individual chromosomes cannot be distinguished; vacuoles have pushed chromatin into corner of cell. $\times 1,000$.—Fig. 26. Immediately after irradiation; anaphase has Y shaped bridge formed by the fusion of ends of daughter chromosomes; this bridge and bridge at right of figure probably resulted in a rupture of the upper group of chromosomes into two parts. $\times 1,100$.—Fig. 27. One hour after irradiation; metaphase chromosomes split and loosely coiled; no spindle is present, chromosomes scattered in the cytoplasm. $\times 1,340$.—Fig. 28. One hour after irradiation; anaphase with many chromosomal bridges. $\times 1,100$.—Fig. 29. One hour after irradiation; anaphase with clockwise deviation of about 60 degrees of lower daughter chromosome group; lateral bridge causing this now broken. $\times 1,050$.—Fig. 30. One hour after irradiation; anaphase in which a single chromosome has adhered to one of opposite group and been pulled away from its normal position. $\times 1,000$.—Fig. 31. Two hours after irradiation; ana-

roots were allowed to grow in water in the dark for ten days. No branch roots appeared. But some seedlings were left in a covered Petri dish and the roots became dry in spots and were moldy. In seven days two branch roots had appeared about 50 mm. back from the tip of one root that had become dry.

The data from these experiments were too meagre to permit an interpretation.² Three possible explanations suggest themselves; the formation of branch roots may have been a response to (1) failure of growth in the tip due to injuries, (2) activity of wound hormones, (3) cessation of formation of growth substance in the tip and, therefore, no inhibition to the formation of branch roots.

Pekarek (1927) reported the presence of groups of meristematic tissue in the middle of the zone of elongation of X-rayed roots of *Vicia faba* fixed three to four days after irradiation. Although at first glance these groups resembled the "Röntgen tumors" seen by Komuro (1925), Pekarek traced their origin to the pericycle and postulated that they represented a premature formation of accessory roots.

The very early branch roots seen in the material from the present experiments exhibited a striking resemblance to the meristematic groups in Pekarek's figures.

ABNORMALITIES IN THE DIVISION FIGURE.—Coincident with the decline in the frequency of nuclear division, a number of abnormalities of division were observed. These were similar in all respects to those

² Patton and Nebel (1940) reported increased formation of branch root initials in cut onion roots after treatment with methylcholanthrene. Elongation, respiration, and dipeptidase activity were decreased in excised corn roots. In the present work, it was found that barley roots treated with neutral red (1:20,000 non-irradiated) showed normal dipeptidase content, whereas irradiated roots (1:75,000 neutral red) showed a marked decrease in enzyme. Patton and Nebel obtained no photodynamic effect of carcinogenic substances using corn roots. Neutral red (Patterson, 1941) was also found to be photodynamically inactive on corn roots.

phase with bridges; several chromosomes in a group separated from one daughter plate. $\times 1,000$.—Fig. 32. One hour after irradiation; chromosomal bridge in anaphase causing complete separation of group of chromosomes from main group in lower daughter plate. $\times 1,165$.—Fig. 33. One hour after irradiation; anaphase with many chromosomal bridges causing separation of daughter chromosomes into three groups resembling those of a tripolar figure. $\times 1,500$.—Fig. 34. Two hours after irradiation; anaphase in which the many chromosomal bridges have caused a separation of daughter chromosomes into four groups giving the appearance of a tetrapolar figure. $\times 1,100$.—Fig. 35. One hour after irradiation; partial pycnosis in anaphase; group of chromosomes form pycnotic mass at equator of division figure, while some of the daughter chromosomes have reached poles; bridges join these with the central mass. $\times 1,200$.—Fig. 36. Two hours after irradiation; partial pycnosis of anaphase chromosomes. $\times 1,100$.—Fig. 37. Two hours after irradiation; late anaphase with strong bridges involving several chromosomes. $\times 1,100$.—Fig. 38. One hour after irradiation; bridge in early telophase; chromosome shows beaded appearance; remaining chromosomes in lower plate are in next section. $\times 1,000$.—Fig. 39. Two hours after irradiation; telophase in which chromosomes in bridge as well as in daughter nuclei are in the spireme condition. $\times 1,000$.—Fig. 40. Three hours after irradiation; pseudoamitosis with nuclei in resting condition and a chromosome in the connecting stalk. Vacuoles prevent normal formation of cell plate. $\times 1,500$.—Fig. 41. Thirty-four and one-half hours after irradiation in experiment IV; Feulgen preparation; pseudoamitosis with stalk containing cytoplasm but very little chromatin. $\times 1,500$.—Fig. 42. Twenty-eight hours after irradiation in experiment IV; Feulgen preparation; late telophase pseudoamitosis with thin chromatic stalk to one side of figure causing deviation of upper daughter nucleus, part of which lies below plane of photograph. $\times 2,350$.—Fig. 43. Twenty-eight hours after irradiation in experiment IV; Feulgen preparation; upper cell contains bridge in telophase involving many chromosomes and probably leading to a pseudoamitosis with unilateral retraction, i.e., more chromatin occurring in upper daughter nucleus than lower. Binucleate cell below must have resulted from pseudoamitosis with thin stalk on right side of figure causing deviation of upper nucleus. $\times 1,500$.—Fig. 44. Nine hours after irradiation; two large dermatogen cells are probably didiploid giant cells arising from pseudoamitosis with bilateral retraction of chromatin due to strong bridges; note large vacuoles. $\times 525$.

described by other workers in tissue subjected to a variety of injurious stimuli. In so far as the abnormalities all occurred in the period before the mitosis-free interval, they may be designated as belonging to the period of "primary effect" of Politzer (1934) or Alberti and Politzer (1923). New divisions, however, were starting during this interval, and abnormalities in these cells should be compared with the "S-mitoses" of Pekarek (1927). This investigator classified abnormal divisions ("P-mitoses") under "primary effect" when the nuclei were in division at the time of X-irradiation, and under "secondary effect" ("S-mitoses") when they were in interphase during irradiation.

In the present work it was difficult, if not impossible, to determine whether a cell was in division at the time of irradiation. The dye probably remained in the cells of the root tips so that the stimulus lasted over a period of time longer than in the case of X-irradiation. The abnormalities appearing immediately after irradiation with sunlight were the same as those appearing later.

Table 1 gives a summary of the abnormalities found in the roots irradiated in a 1:75,000 solution of neutral red for one hour in sunlight. The pro-phases appeared to be normal in all the material and were not included in the table. The data were taken from experiment I except as otherwise noted. No abnormalities of the degree recorded here were seen in any of the controls.

The frequency of cell division in root tips fixed immediately after irradiation remained high. Abnormalities appeared in divisions near the periphery of the root. A typical pycnotic metaphase in a cell of the dermatogen lying about .5 mm. up from the tip is shown in figure 25. The individual chromosomes cannot be distinguished in the densely staining mass from which their ends project. This pycnosis is the same as that described by Alberti and Politzer (1923) in X-rayed corneas of salamander larvae. In

the irradiated barley roots it occurred in 3 per cent of the metaphases and was the only abnormality of this stage seen immediately after irradiation. Eighteen per cent of the anaphases were abnormal, half of these occurring in cells of the dermatogen. The ends of the daughter chromosomes were fused, forming such strong bridges that during the anaphase movement part of the entire group of chromosomes at one pole was dragged away from the rest (fig. 26, center cell; note Y-shaped bridge). Thus it might be said that the presence of these bridges had caused a separation of the chromosomes in the normally compact daughter plate. This could be called a partial deviation (Alberti and Politzer, 1923, p. 94) in that a portion of the daughter group of chromosomes was pulled away from its normal orientation by the chromosomal bridge. (This phenomenon is further illustrated in figures 29-34 from material fixed one and two hours after irradiation.) Three per cent of the telophases had bridges, and a pseudoamitosis was present with an incomplete cell plate. The fact that seven binucleate cells were found, four in the dermatogen, would seem to indicate either that the formation of the cell plate had been affected, or that earlier pseudoamitoses had a stalk-thinning (Stielverdünnung) end stage (Politzer, 1934) which resulted in a binucleate cell.

Material fixed one hour after irradiation showed a much higher percentage of abnormality of division. Aberrant mitoses were scattered throughout the root tip. Forty-four per cent of the metaphases were abnormal. Half of these were pycnotic and the other half had split, coiled chromosomes scattered in the cell (fig. 27). This latter phenomenon was described by Marquardt (1938) as occurring in somatic mitoses in the root tips of *Scilla campanulata* after treatment with soft X-rays. In the present experiments, it might be possible that cells exhibiting this failure of spindle formation were in interphase at the time of irradiation.³ Their occurrence was much less frequent in the material fixed later in experiment I. In roots fixed two hours after irradiation in experiment IV, 81 per cent of the metaphases were in this condition. Apparently the cycle was stopped here because in the 28- and 34½-hour material these scattered chromosomes were disintegrating. The pycnotic metaphases also disintegrated. This disintegration was not limited to material in experiment IV. In

³ While this manuscript was in the course of preparation, a film was seen, made by Dr. Margaret Lewis, showing dividing tissue culture cells growing in media containing fluorescent substances, one of which was neutral red (1:200,000). Two types of injury to the cells resulted from irradiation by the light used for taking the motion pictures. If a cell was exposed just before anaphase, the chromosomes separated and went to opposite poles, although they frequently stuck to each other forming bridges, resulting often in one daughter nucleus receiving more chromatin than the other. If a cell was exposed before metaphase, the chromosomes lined up on the spindle which then "softened and shortened" finally dissolving and freeing the split, sometimes clumped, chromosomes in the cytoplasm. The cells died when exposed to the photodynamic influence too long.

all the experiments pycnotic metaphases often occurred in cells of the dermatogen that were about to die. The arrest in metaphase was permanent since the cells in which it occurred were dying. Stages of division were seen that resembled the pycnotic metaphases described by Pauli and Politzer (1929) as present in the corneas of salamander larvae after irradiation with cathode rays. In living cells all metaphases continued to anaphases in the present work.

The longer the interval between irradiation and fixation of the root tips, the greater was the percentage of abnormalities in all the stages of division. In experiment I, anaphases were particularly interesting in material fixed one and two hours after irradiation. In sections of this material 50 per cent and 75 per cent, respectively, of the anaphases showed chromosomal bridges (fig. 28). In half of these anaphases the bridges were stronger on one side than on the other so that deviation of the daughter chromosomes occurred (fig. 29, bridge just broken). There must have been an actual adherence of the chromosomes in these bridges. An anaphase in which a single chromosome has been pulled away from its normal position in the daughter group is shown in figure 30. Anaphases can also be seen (fig. 31, 32, 33, 34) in which several chromosomes in a group have been separated from a daughter plate. In every case the bridges instrumental in causing this partial deviation were evident. A cell with two bridges and a separation of the daughter chromosomes into three groups is seen in figure 33. Figures that might easily be confused with tripolar (fig. 33) or tetrapolar (fig. 34) divisions arose in this way. Either only one daughter plate was pulled apart into two groups or both daughter plates were separated.

In some cases partial pycnosis of the anaphase chromosomes was seen. This phenomenon is the same as that described by Alberti and Politzer (1923, p. 98, fig. 14). A group of chromosomes formed a pycnotic mass at the equator, while two small groups of daughter chromosomes were at the poles (fig. 35, 36). Bridges extended between the chromosomes at the poles and the central mass. Patten and Wigoder (1930) gave similar figures ("gummy mitoses") from X-rayed bean root tips. These occurred during the recovery period.

In the present work it was evident that some of the bridges persisted through late anaphase (fig. 37) and telophase (fig. 38). The chromosomes in the bridge in the latter figure showed a beaded appearance. The remaining chromosomes of the lower plate were seen in the next section. The presence of these bridges led to the formation of pseudoamitotic figures. A cell in which the chromosomes in the bridge as well as in the daughter nuclei were in a telophase spireme is shown in figure 39. Sometimes cells were seen with the daughter nuclei in the resting condition but with a chromosome in the bridge (fig. 40). The sections of root tips showed all three types of end stage of pseudoamitosis described by Politzer (1924) as occurring in corneas of salamander larvae

in non-irradiated solutions of neutral red. As seen from table 1 the stalk-thinning (*Steilverdünnung*) end stage was the most common in root tips. A thin stalk connected the two daughter nuclei (fig. 41 and 42, Feulgen preparations). Sometimes chromatic material was present in the stalk (fig. 42); at other times it was composed only of a clear material (fig. 41). The connection between the daughter nuclei became progressively thinner and finally ruptured giving rise to a binucleate cell (fig. 43). Often the connection occurred very much to one side of the cell (fig. 42, part of upper daughter nucleus below plane of photograph; Feulgen preparation). This gave rise to a binucleate cell in which the daughter nuclei showed great deviation from their normal position with reference to each other (fig. 43).

When the bridges involved a great many chromosomes, the other end stages of pseudoamitosis appeared. In cases of unilateral retraction (fig. 43, Feulgen preparation), the daughter nuclei were of unequal size. Sometimes one was chromatin-free. With bilateral retraction, a didiploid giant cell arose (fig. 44). Out of 102 pseudoamitoses observed, 86 per cent showed evidence of stalk-thinning, 7 per cent bilateral retraction, and 7 per cent unilateral retraction. The fact that a number of binucleate cells were found supports these results.

Many of the binucleate cells, however, may have arisen merely because of lack of formation of a cell plate. The cells were often so full of vacuoles that the plate presumably could not be completed. Becker (1929, 1930) found the same types of abnormalities as those described above in onion root tips grown in solutions of methylene blue or neutral red. He reported that the division membrane was frequently partially or completely missing and that binucleate cells were formed. Becker's (1933) observations on the effect of dye solutions on cell division in the stamen hair cells of *Tradescantia virginiana* showed that vacuoles often interfered with formation of the cell plate. Neutral red was found to be injurious only in very concentrated solutions (1:500). The daughter cells were seen to be joined by bright, structureless strands which divided the phragmoplast. Becker (1935) stained these cells vitally with chrysoidin or Bismark Brown and followed the behavior of the spindle. No trace of formation of a cell plate could be observed. The phragmoplast disappeared, leaving a typical binucleate cell.

It may be concluded, therefore, that the photodynamic action of neutral red on the root tips of barley seedlings produced typical abnormalities of nuclear division. These consisted of pycnosis, formation of bridges in anaphase, and pseudoamitosis. The usual end stage of the pseudoamitoses was that of stalk-thinning (*Stielverdünnung*), although asymmetrical and bilateral retraction end stages were observed. Binucleate cells were formed. This material gave evidence that the anaphase bridges were not due to a simple lagging of the chromosomes as described

by Pekarek (1927), but that an actual adherence of their ends had occurred (Politzer, 1934; Marquardt, 1938).

DISCUSSION.—Since the early work on the photodynamic action of fluorescent dyes (v. Tappeiner, 1900) it has been known that irradiation with visible light greatly increased the toxic effect of these dyes. Irradiation of a cell or an organism in a dilute solution of the dye gave results similar to those produced by a concentrated solution of the dye in the dark.⁴

The photodynamic action of neutral red on the eggs of the sea urchin, *Lytechinus variegatus*, has been studied by Tennent (1935, 1938a, b) in the course of an extensive investigation of the photodynamic action of dyes. Solutions of neutral red in sea water (1:150,000) were toxic to cultures irradiated in sunlight three minutes or more.

The wave lengths of light that were effective lay in the blue end of the visible spectrum. Using a General Electric exposure meter and calibrated filters, Tennent (1938a) found that the "threshold for violent surface reaction (blister cytolysis) of *Lytechinus* eggs in neutral red sea water lies at about 2,500 foot candles."

Tennent (1938b) reported that dividing eggs in solutions of neutral red irradiated in sunlight for two to eight minutes completed their division but showed abnormalities characteristic of the primary effect after X-irradiation, i.e., lagging and pycnosis of chromosomes. He states: "It would be possible to make a phase by phase comparison of the photodynamic effect of neutral red on cell division with the phases obtained by Politzer and others after X-ray irradiation" (p. 101).

Comparison of the effect of X-rays with the toxic action of neutral red alone on the corneas of salamander larvae (Politzer, 1934) shows that the abnormalities found before the mitosis-free interval differ in frequency but not in kind. The great difference lies in the fact that mitoses occurring after the mitosis-free interval are completely normal after neutral red, whereas after X-ray treatment abnormalities are seen for weeks. These include fragmentation of the chromosomes, their resultant abnormal distribution, and formation of accessory nuclei.

Similar conclusions result from a comparison of the photodynamic effect of neutral red as found in the present experiments with the effect of X-rays on plant root tips (Pekarek, 1927; Bersa, 1927; Patten and Wigoder, 1930; Marquardt, 1938). Pekarek (1927) gave an exhaustive description of the effect of X-rays on the root tips of *Vicia faba*. The fall in frequency of cell division, maturing of the tissue, formation of branch roots, and occurrence and nature of abnormalities of division before the mitosis-free interval were very similar to the phenomena found due

⁴ It is now clear (see Blum, 1941, page 83) that the mechanism of photodynamic action differs essentially from that of "dark action." But as far as the resulting abnormalities of nuclear division are concerned, the end effect of the two actions seems to be similar.

to the photodynamic action of neutral red.⁵ In the case of X-irradiation, however, new divisions showed typical fragmentation, etc., while they were normal after neutral red.

Bersa (1927) found that the root tips of *Zea mays* were very resistant to the effect of X-irradiation. The frequency of cell division fell slowly reaching a minimum at 24 hours. Even with the largest doses (16 H), no mitosis-free interval was seen. Abnormalities of nuclear division first occurred in material fixed 36 hours after irradiation. They were not so numerous as those described by Pekarek (1927) or Alberti and Politzer (1924). The number of prophase fell, then rose, but otherwise the division cycle was normal.

Every tissue response described as occurring in root tips of barley seedlings that had been irradiated in a 1:75,000 solution of neutral red, has been found in plant material subjected to other stimuli. Therefore in this material the photodynamic action of neutral red is non-specific in character.

SUMMARY

Roots of barley seedlings were irradiated for one hour in sunlight in a 1:75,000 solution of neutral red and subsequently grown in water in the dark. Control roots in dye solution in the dark and control roots irradiated in distilled water showed no abnormalities. Sections of the experimental roots fixed at va-

⁵ A paper by Prescher (1932) on the photodynamic action of eosin on root tips of *Vicia faba* has recently come to the author's attention. Counts of cell divisions were made on roots grown in light in eosin solutions (1:5,000, 1:10,000, 1:25,000, 1:100,000) and on complete sets of controls. The results are in general in agreement with the present work, with the exception that Prescher found no mitosis-free interval, no bridges in anaphase, and no pseudoamitoses. If a concentration of eosin between 1:25,000 and 1:100,000 had been used, these abnormalities might have been observed.

rious intervals after irradiation showed many abnormalities in the cells and tissue. Immediately following irradiation, vacuolization of the dermatogen cells of the root tip set in. Along with a rapid fall in frequency of cell division, the vacuolization reaction spread throughout the meristem region, increasing in intensity so that twenty-three hours after irradiation the entire root tip contained cells typical of the zone of elongation of the root. The root tips were mitosis-free after seven hours.

Roots observed 20 to 70 hours after irradiation in dye solution exhibited marked ageotropic bending. Study of the sections showed that in every case a greater number of dead cells were present on the concave than on the convex side of the root. The side facing the sun was photodynamically injured, and the bending was probably due to a contraction of the dead cells or a greater elongation of the cells which were shaded, or both. Formation of branch roots in the bend of the main roots was often observed. These roots grew toward the convex side of the main root. Instances of this were also found in injured roots of the controls.

The abnormalities in cell division occurring before the mitosis-free interval consisted of pycnosis, formation in anaphase of bridges, and pseudoamitoses. The stalk-thinning end stage of pseudoamitosis was the most frequent, although cases of unilateral and bilateral retraction were seen. These abnormalities, together with the fall in frequency of cell division, maturing of the tissue, and formation of branch roots, are similar to some of the effects found by others after X-irradiation of plant tissue, as well as after other stimuli.

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INHIBITORY EFFECTS OF INORGANIC COMPOUNDS ON PHOTOSYNTHESIS IN CHLORELLA¹

Sydney S. Greenfield

WARBURG'S (1919, 1920) demonstration that cyanides affect the dark reactions, and urethanes the light reactions in photosynthesis has been useful in research and theoretical discussions. Since earlier work had shown that inorganic salts were capable of depressing photosynthesis in *Elodea* (Trebois, 1903; Pantanelli, 1903) and other plants (Spoehr, 1926), the present investigation was made to study further the inhibitory effects of several inorganic compounds on photosynthesis in *Chlorella* and to determine, in particular, whether they could separately inhibit the dark and the light reactions. It was thought that the results of this work might provide evidence which would be useful in the study of the mechanism of photosynthesis as well as of the physiological influence of these chemicals.

The present paper reports the effects of excessive concentrations of certain chemical substances, most of which have been demonstrated as necessary in plant nutrition, on the rate of photosynthesis in *Chlorella vulgaris*. The compounds employed include some known to be toxic in very low concentrations and others only effective at much higher concentrations. Sucrose solutions were used to determine which salts act through an osmotic effect and to study this effect. Rates of photosynthesis were measured by means of Warburg manometers. In order to determine the relations of the inhibitors to the light and dark reactions in photosynthesis, inhibition was studied at five light intensities, over a range from where light was the limiting factor to where it was in excess. A preliminary note on some of the results has appeared in *Science* (Greenfield, 1941).

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The experiments were performed in the laboratory of plant physiology at Columbia University. The author is indebted to Professor Sam F. Trelease for suggestions and criticisms throughout the course of the investigation and the preparation of the manuscript.

MATERIALS AND METHODS.—Tests were made with the unicellular alga *Chlorella vulgaris*, cultured according to the method of Warburg (1919) as modified by Craig and Trelease (1937). Culture flasks of liter volume were used with 300 cc. of the following solution, KNO₃, 0.025 M; MgSO₄, 0.020 M; KH₂PO₄, 0.018 M; FeSO₄, 0.00001 M; K-citrate, 0.00001 M; and 14.2 cc. per liter of a stock solution containing the microtrophic elements boron, copper, manganese, zinc, etc. (Trelease and Trelease, 1935). A mixture of 5 per cent CO₂ in air was continuously bubbled through the culture solutions, and a 300-watt tungsten filament lamp, cooled by a water jacket, was the light source. The flasks were kept 15 cm. from the lamp, where the average light intensity was about 10,000 lux. New cultures were inoculated with 35 million cells from an actively growing culture, and populations of 18 to 25 million cells per cc. were obtained on the fourth day. At this stage of active growth, cells were removed from the cultures for experiments and for new culture inoculation. Cell concentrations were estimated with a haemocytometer.

Since the purpose of this study was to analyze the effects of chemicals caused by their presence during rate measurement and not in culture, it would have been desirable to dissolve the salts in the test buffer. As several salts caused an evolution of gas from the carbonate-bicarbonate buffer selected, this was not feasible, but left the choice of either using another test medium or employing a method of cell treatment and washing prior to suspension in the buffer. After preliminary tests, the latter choice was made, to permit rate measurements of control and treated cells under the uniform external conditions obtainable in the buffer. The method of pretreatment eliminated reactions of the test substances with the suspension medium that might have altered gas solubilities or

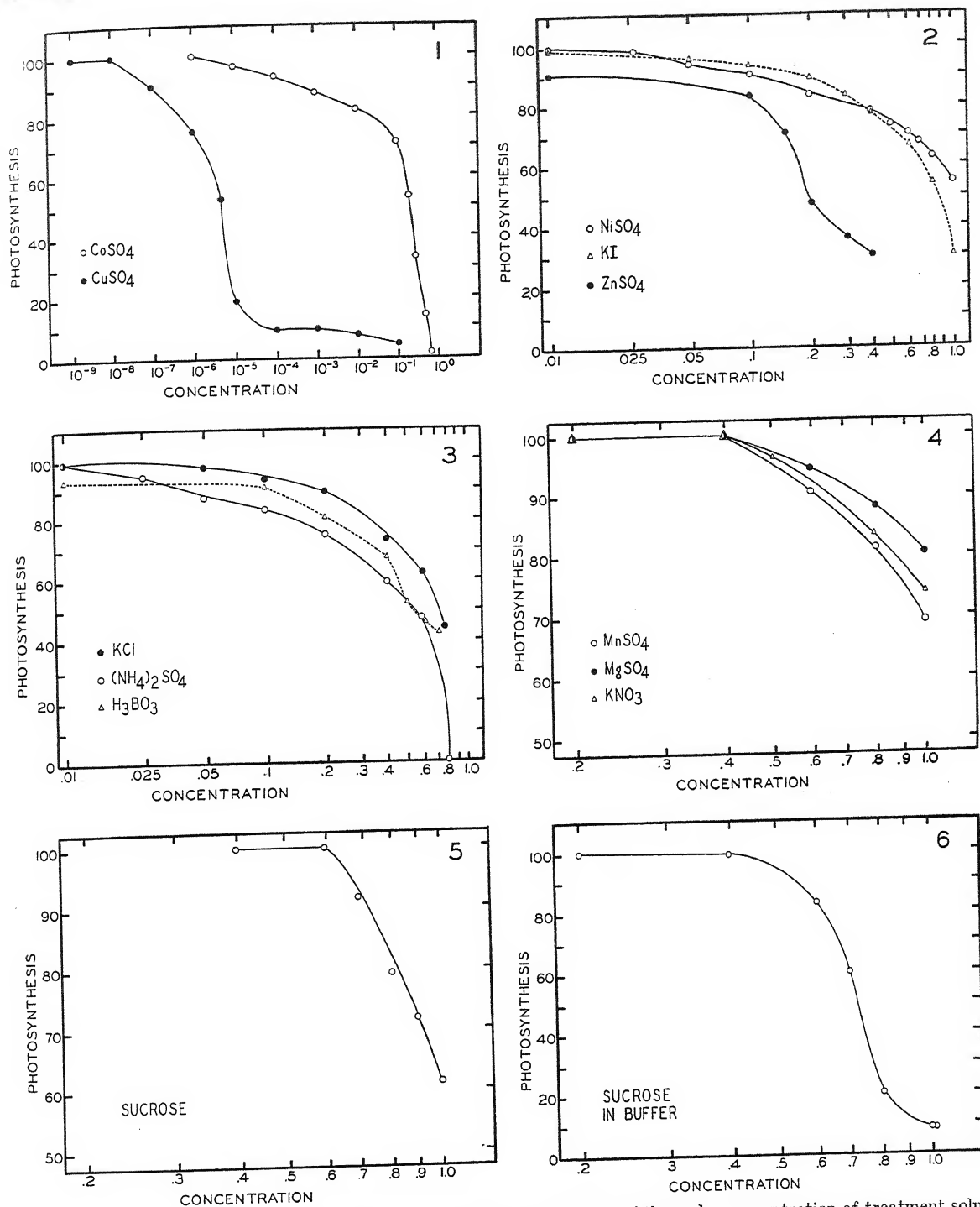


Fig. 1-6. Rates of photosynthesis (percentage of control) as a function of the molar concentration of treatment solution. Logarithmic concentration scale. Light intensity 22,000 lux.

factor, suspensions were kept in the dark during treatment.

The results of these experiments are shown in tables 1 to 3, wherein the rates given are the averages of several determinations. The relationships between rates of treated and untreated cells are plotted

in figures 1-4, using logarithmic scales of concentrations to avoid crowding of points.

The inhibitory effect of CuSO_4 was most pronounced, being evident at the low concentration of 10^{-7} M (fig. 1) and increasing strongly at higher concentrations. Solutions of CoSO_4 (table 1, fig. 1)

[illegible]

effective below the threshold of osmotic pressure inhibition and, therefore, any chemical effect they may have had on the photosynthesis of *Chlorella* could not be detected.

Calculations for all of the substances previously considered (fig. 1-3) showed them to be effective below the point of interference by osmotic pressure, as determined by the experiments with sucrose. They are consequently capable of an inhibition independent of this factor. Although in some cases the curves extend into the region of osmotically active concentrations, they exhibit no secondary drop, such as might be expected. The effect of the withdrawal of water was not observable, since it may have been masked by the chemical inhibition. Although the water content was reduced, it did not become the limiting factor.

Respiration.—The respiratory rates in almost all of these experiments were similar. They are not listed separately, because, although they are sufficiently reliable for correcting the apparent photosynthetic rates, they are less precise than the latter, due to the small readings obtained. The rates averaged about 0.07 c.mm. of O_2 consumed per minute by the standard sample of 35 million cells. At high light intensities the respiration rate was less than 5 per cent of the photosynthetic rate; but, at the lowest light intensities used in experiments described later in this paper, it was as much as 30 per cent. Treatment with high concentrations of $CoSO_4$ and $CuSO_4$ slightly depressed respiration. With 0.8 M and 1.0 M $(NH_4)_2SO_4$, which completely stopped photosynthesis, the respiratory rate was normal. Inhibition of photosynthesis in normally respiring cells was first observed by Bernard (1878), and the observation has been confirmed (Irving, 1911; Spoehr, 1926).

TABLE 4. *Respiration rates (c.mm. O_2 absorbed by 35 million cells per minute) of cells treated with $HgCl_2$. The rates are averages of two or three experiments wherein both light and dark rates were determined with the same cells. Light intensity 22,000 lux.*

$HgCl_2$ molarity	Light rate	Dark rate	Ratio: Light/dark rates
2.5×10^{-5}	0.125	0.065	1.9
5×10^{-5}	0.140	0.070	2.0
10^{-4}	0.155	0.075	1.9
10^{-3}	0.145	0.070	2.1
10^{-2}	0.160	0.050	3.2
10^{-1}	0.075	0.045	1.7

Treatment with concentrations of $HgCl_2$ of 2.5×10^{-5} M or higher completely inhibited photosynthesis, but consumption of oxygen in respiration was observed in the light as well as in the dark. Although other respiratory data are incorporated into the corrected photosynthetic rates, those determined in the experiments with $HgCl_2$ merit separate presentation. Control rates in the light were obviously unobtainable, but in the dark they were about

0.07 c.mm., the same as those of treated cells, in most cases. Table 4 gives the rates in the light and dark for each concentration which completely inhibited photosynthesis.

The respiration rate in the dark was normal except with the two highest concentrations, but in the light the rate was approximately doubled in most cases. Whether this high respiratory rate in the light is normal for illuminated cells, or due to $HgCl_2$ treatment, cannot be determined from these experiments. Poisons are known to accelerate metabolic processes temporarily (Reynolds, 1939), but since photosynthesis is here completely inhibited, it may be the true respiratory rate in the light that is revealed. Van der Pauw (1932) was able to stop photosynthesis in *Horridium* by treatment with 0.002 M KCN and also observed a higher respiration rate in the light than in the dark.

Recent investigations have suggested the presence of a photo-oxidation in the photosynthetic system, since large amounts of carbon dioxide were observed to evolve during the induction period (Gaffron, 1940) and during measurements at injurious light intensities (Myers and Burr, 1940). In the present experiments the rapid oxygen absorption by cells pretreated with $HgCl_2$ might be related to a photo-oxidation, but such an interpretation would be premature with the information now available.

Effects on subsequent culture.—In order to study further the effects of the substances found to inhibit photosynthesis in relation to behavior of cells in culture, to duration of treatment, and to light intensity, a single concentration of each substance was selected. Wherever possible, a concentration was chosen which caused approximately 50 per cent inhibition of photosynthesis at 22,000 lux. In other cases a less depressing concentration had to be selected, below the critically effective osmotic pressure threshold, in order to insure the study of the effect of the substance itself, uncomplicated by the reduction of water content in the cell. These concentrations are indicated in the experiments, and are summarized in table 5:

TABLE 5.

Substance	Concentration reducing photosynthesis to about 50 per cent of the control	Concentration used in subsequent experiments
$CuSO_4$	5×10^{-6}	5×10^{-6}
$CoSO_4$	0.2	0.2
$ZnSO_4$	0.2	0.2
H_3BO_3	0.5	0.5
$(NH_4)_2SO_4$	0.5	0.4
KCl	0.7	0.3
KI	0.8	0.3
$NaSO_4$	1.0	0.4

For each substance an experiment was performed to test the effect of treatment with the selected con

centration on subsequent culture. Cells were treated in the regular manner for twenty minutes and then washed, but instead of being suspended in the buffer, 35 million cells of the treated group were inoculated into 300 cc. of culture solution and an equal number

The effects of treatment with HgCl_2 on subsequent growth were also studied. Three flasks of culture solution were inoculated with 35 million cells of groups treated with distilled water, 10^{-5} M, and 10^{-4} M HgCl_2 , respectively, and were observed for five days. Throughout this period, the group treated with

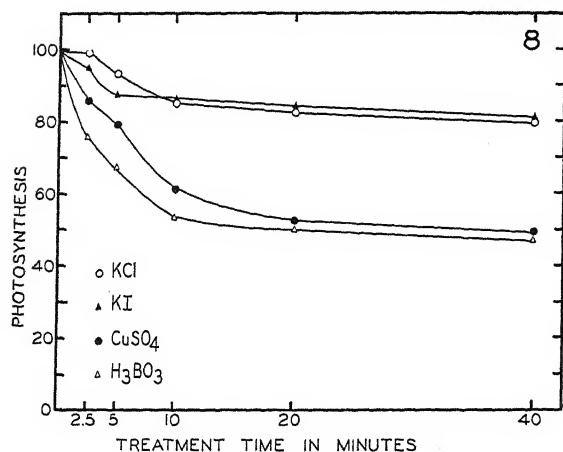
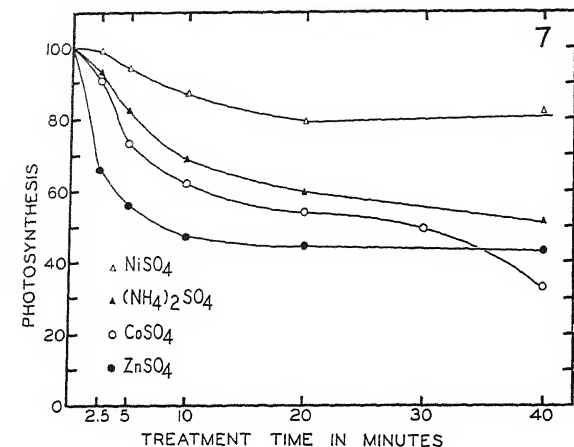


Fig. 7-8. Rate of photosynthesis (percentage of control) as a function of treatment duration in minutes. Following the treatment, 10 minutes were required for centrifugation and decantation.

of cells treated with distilled water was inoculated into another culture flask. Both were then grown under the standard culture conditions described, and compared daily for five days.

The results of all tests were essentially the same, except for the cells treated with CoSO_4 , where a slightly lower cell count was evident. Cell numbers, greenness, size, and general appearance were similar in cultures inoculated with untreated and treated cells. Thus plants subjected to a treatment which inhibited photosynthesis as much as 50 per cent appeared to grow and reproduce normally when inoculated into a culture solution. The treatment seems to have produced a state wherein there was present in the cell a concentration of the inhibiting substance which affected photosynthesis without causing permanent alteration in the cell systems.

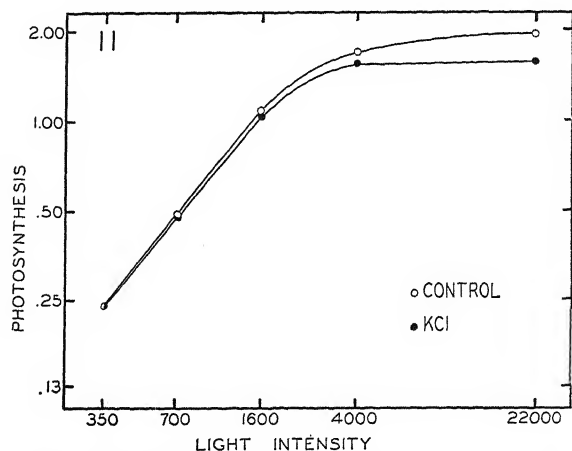
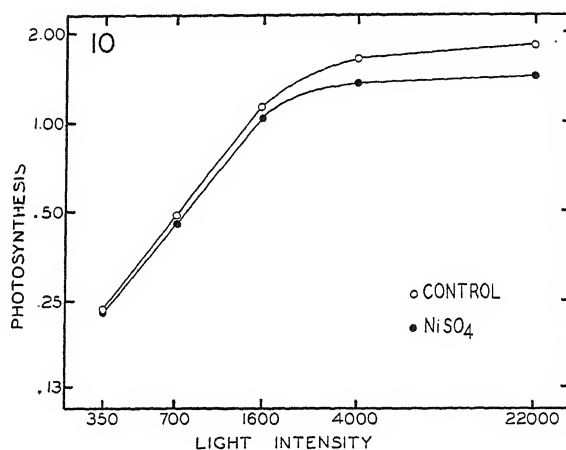
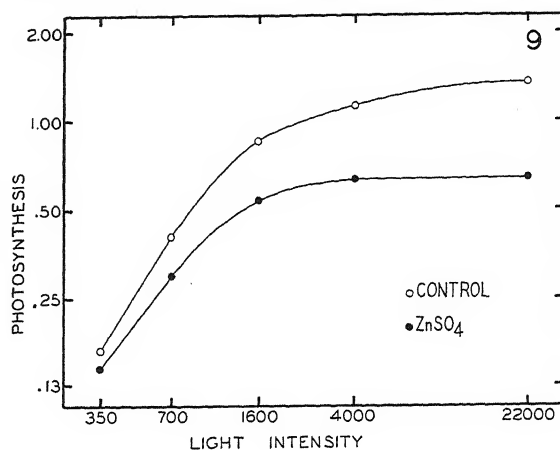


Fig. 9-11. Rate of photosynthesis (c.mm. O_2 per min. per 35 million cells) for treated cells and controls. Light intensities in lux. Both scales logarithmic.

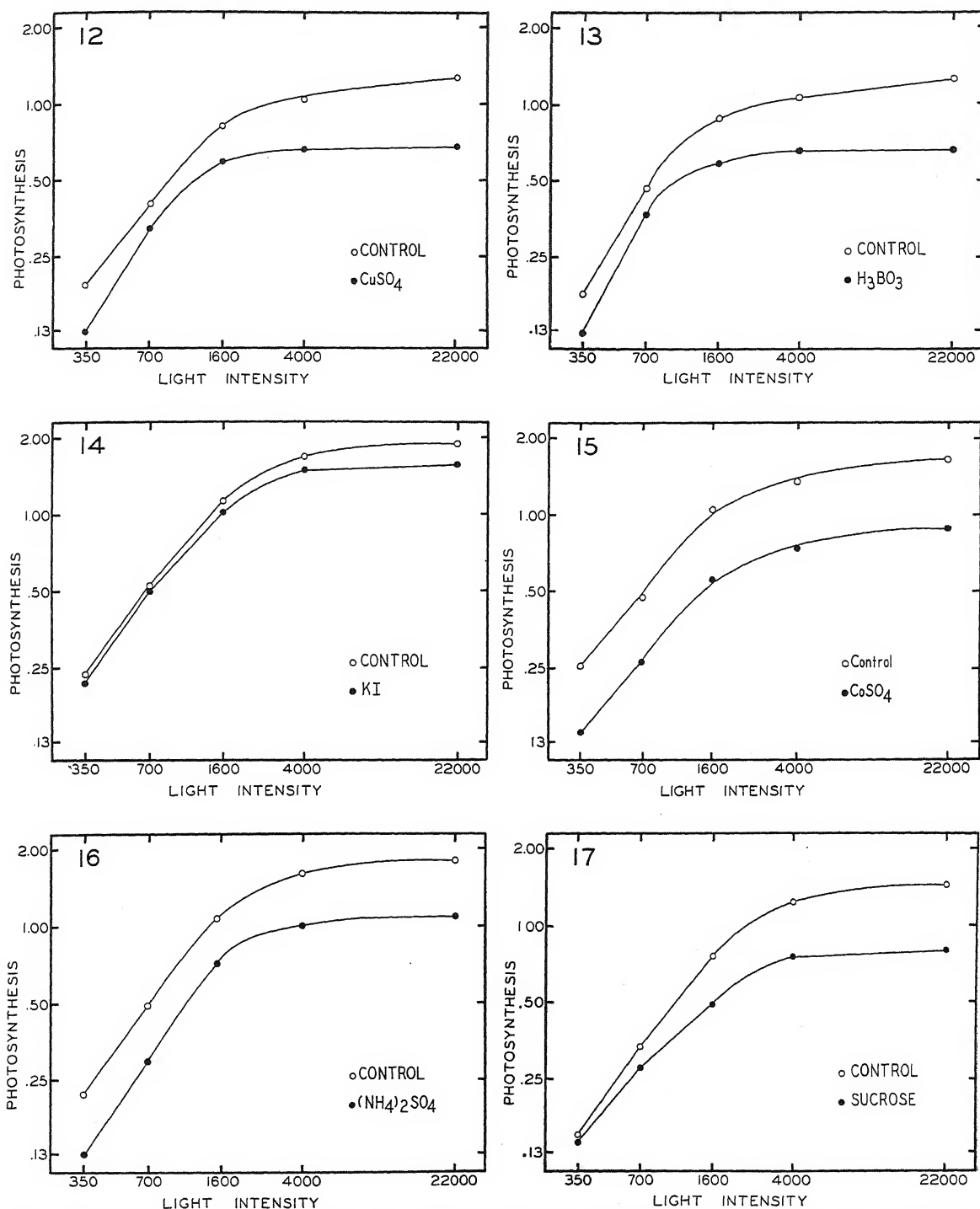


Fig. 12-17. Rate of photosynthesis (c.mm. O_2 per min. per 35 million cells) for treated cells and controls. Light intensities in lux. Both scales logarithmic.

10^{-5} M HgCl_2 grew like the control culture, indicating no residual toxic effect of the treatment. However, the cells treated with 10^{-4} M HgCl_2 were pale green when introduced into the culture solution, and the culture remained pale for three days. During this time the cell population did not appreciably increase,

nor did the chlorophyll return to normal color. On the fourth day, however, the culture was definitely green, and considerable multiplication had occurred. It exhibited normal growth thereafter. The treatment did not kill all the cells, since subsequent growth, although delayed, was evident. HgCl_2 ap-

pears to have stopped photosynthesis temporarily by injuring the cells and destroying some of the chlorophyll. After sufficient time had elapsed for recovery (involving possibly some outward diffusion of the toxic salt) and production of new pigment, the cells functioned normally.

TABLE 6. Rates of photosynthesis (c.mm. O_2 per min. per 35 million cells) at five light intensities, for untreated cells and cells treated with test solutions or in a buffer with sucrose.

Test solution		Light intensity in lux				
		350	700	1,600	4,000	22,000
$ZnSO_4$ 0.2 M	Control	0.168	0.400	0.853	1.118	1.343
	Treated	0.145	0.300	0.540	0.630	0.638
$NiSO_4$ 0.4 M	Control	0.238	0.490	1.140	1.648	1.803
	Treated	0.230	0.438	1.038	1.363	1.435
KCl 0.3 M	Control	0.240	0.488	1.090	1.670	1.925
	Treated	0.243	0.473	1.038	1.565	1.583
$CuSO_4$ 5×10^{-6} M	Control	0.195	0.430	0.815	1.035	1.260
	Treated	0.135	0.340	0.585	0.660	0.675
H_3BO_3 0.5 M	Control	0.180	0.470	0.885	1.065	1.253
	Treated	0.128	0.370	0.593	0.660	0.660
KI 0.3 M	Control	0.230	0.525	1.135	1.665	1.885
	Treated	0.210	0.495	1.023	1.495	1.580
$CoSO_4$ 0.2 M	Control	0.256	0.498	1.068	1.344	1.650
	Treated	0.140	0.264	0.558	0.738	0.876
$(NH_4)_2SO_4$ 0.4 M	Control	0.220	0.488	1.073	1.590	1.800
	Treated	0.130	0.300	0.720	1.005	1.095
Sucrose 0.7 M in buffer	Control	0.153	0.338	0.755	1.230	1.440
	Treated	0.140	0.278	0.490	0.758	0.795

Influence of treatment duration.—Experiments in which the time of treatment was varied were performed with the selected concentration of each inhibitory substance. The time periods used were 2½, 5, 10, 20, 30, and 40 minutes, plus 10 minutes required for centrifugation and decantation of the test solutions. The amount of inhibition is plotted as a function of time of treatment in figures 7 and 8. The results of these tests indicate that the twenty-minute period used in most of the experiments (twenty minutes of treatment plus ten minutes of centrifuging and decanting) was expedient, since the leveling of the curves indicates that a state of equilibrium was reached before the end of the time period chosen (fig. 7, 8). This equilibrium is not a simple penetration equilibrium, since it also involves diffusion activity, following the soaking period, both in the distilled water wash and in the buffer.

The curves for cells treated with $CoSO_4$ and $(NH_4)_2SO_4$ show secondary increases in inhibition after forty minutes' treatment. Not indicated on the curve for $CoSO_4$ tests are values for longer experiments wherein the rate of photosynthesis was reduced to 14 per cent by continuing the treatment for one hour, and stopped completely by extending to three hours. These cells still carried on respiration and showed no abnormal appearance. It is perhaps indicative of like modes of action that the effects

of $CoSO_4$ and $(NH_4)_2SO_4$ are similar in the experiments which follow.

Influence of light intensity.—The rates of photosynthesis of cells treated with inhibitory substances were measured at five light intensities in order to determine their effects on the light and dark reactions. The concept that inhibition of the photochemical or dark reactions may be determined by comparing inhibitory effects at low and at high light intensities has been used by Warburg, Spoehr, Emerson, Arnold, Briggs, and many other investigators. It has been discussed recently in critical reviews by Franck and Gaffron (1941) and by Manning (1938).

The data of the present experiments at various light intensities are given in table 6, wherein the rates recorded are averages of several determinations. In figures 9 to 17 the rates of control and treated groups are plotted as a function of light intensity. Logarithmic scales have been used because they avoid crowding of points at low light intensities and give essentially straight lines for the parts of the curves in the range where light intensity is limiting. [The control curves are similar in type to those

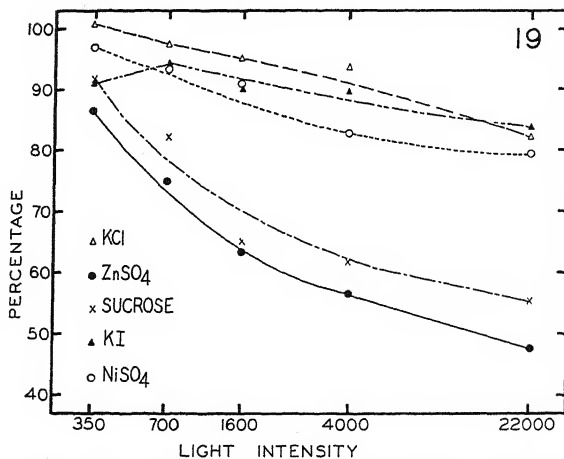
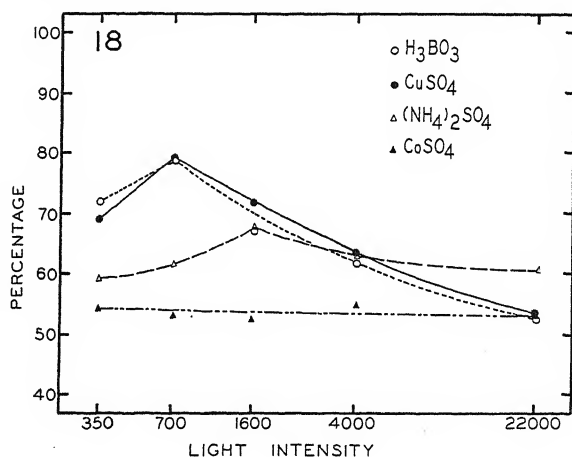


Fig. 18-19. Relative rate of photosynthesis of treated cells (percentage of control rate) as a function of light intensity. Light intensities in lux on logarithmic scale.

of Smith (1937), but they represent different tests and are neither identical with his nor with each other.]

The results may be explained in relation to figure 20, in which the control or normal curve is used as the basis of comparison, and the general types of curves obtained with treated cells are illustrated. The curves are interpreted according to the limiting factor hypothesis of Blackman (1905). Thus, in the normal curve AB the rate of photosynthesis rises with increase in light intensity over the low intensity range, because here light limits, or determines, the rate of the whole process of photosynthesis. But at higher light intensities the curve flattens, since here light is in excess, and some factor controlling the dark reaction limits the rate. In the same sense, at low light intensities the photochemical reaction is limiting, and at high light intensities the dark reaction is limiting.

An inhibition of the dark reaction alone should result in a curve like AED (fig. 20), where inhibition is most evident when the dark reaction determines the rate and diminishes as the limiting factor status of this reaction decreases. The curves of figures 9–11, representing treatment with 0.2 M ZnSO_4 , 0.4 M NiSO_4 , and 0.3 M KCl , resemble AED in figure 20 and indicate that these substances affect only the dark reaction, since the curves of untreated and treated cells are close at low light intensity and diverge as the light intensity increases. This is also illustrated in figure 19, where a continuous drop in relative rates is evident from low to high light intensity for cells treated with ZnSO_4 , NiSO_4 , and KCl . Other studies have shown that the dark reaction may be retarded by heavy water (Craig and Trelease, 1937; Pratt and Trelease, 1938) or by high concentrations of carbon dioxide (Livingston and Franck, 1940).

If a substance affected only the photochemical reaction, greatest inhibition of photosynthesis, as shown by greatest divergence of curves for control and treated cells (as between A and C of curves AB and CEB, fig. 20), would be evident at low light intensities where the photochemical reaction determined the rate, and retardation would become negligible at high light intensities where the dark reaction became limiting and the light reaction was in excess. None of the inorganic substances tested gave a curve like CEB, but phenylurethane in Warburg's (1919) experiments approximated this type.

A substance which retards both photochemical and dark reactions should give a rate curve which resembles the composite curve CED in figure 20. Variations of this curve have been obtained. The effects of 5×10^{-6} M CuSO_4 , 0.5 M H_3BO_3 , and 0.3 M KI may be considered together since they resulted in similar curves (fig. 12, 13, 14). Examination of the curves from 22,000 lux down to 700 lux indicates a convergence and, therefore, an inhibition of the dark reaction; but from 700 lux down to 350 lux the curves diverge, indicating an inhibition of the light reaction. (This is shown also in figure 18, where there is a

drop in relative rate below 700 lux for CuSO_4 and H_3BO_3 tests, and in figure 19 for KI tests.) In figures 12 and 13 the points of closest proximity of control and experimental curves actually lie between 700 and 1,600 lux. These points correspond to the light intensity at which theoretically two separate inhibition curves cross and are resolved into the plotted curve. The curves for cells treated with CuSO_4 , H_3BO_3 , and KI are composites representing chiefly an inhibition of the dark reaction and to a lesser extent an inhibition of the light reaction.

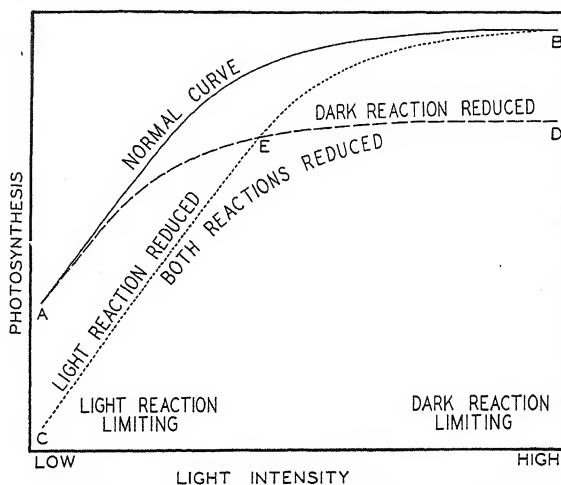


Fig. 20. Diagram of differential inhibition of light reaction and dark reaction. (The normal curve is a composite of all control data plotted on logarithmic scales, and the other curves have been drawn with the same shape.)

Inhibition by 0.2 M CoSO_4 and 0.4 $(\text{NH}_4)_2\text{SO}_4$ results in curves (fig. 15, 16) which are approximately equidistant from the control curve at all light intensities, except that in figure 16 the curves are somewhat closer in the region of 1,600 lux. These curves resemble the composite curve CED in figure 20. In figure 18 it is evident that the amount of inhibition by CoSO_4 was about the same at all light intensities, but for $(\text{NH}_4)_2\text{SO}_4$ there is an indication of least inhibition at 1,600 lux. It may be concluded that CoSO_4 inhibits photosynthesis by retarding both the photochemical and the dark reactions about equally, and that $(\text{NH}_4)_2\text{SO}_4$ approximates this action.

Tests with sucrose solutions, introduced as a basis for determining the osmotic pressure threshold of inhibition, indicated that solutions of KNO_3 , MnSO_4 , and MgSO_4 only inhibited photosynthesis when they were sufficiently concentrated to exert an osmotic effect and lower the water content of the cells. Inhibition by sucrose solutions of high osmotic pressure was studied at various light intensities. Cells were placed in a buffer that contained sucrose in a concentration of 0.7 M. From table 6 and figures 17 and 19 it is clear that the amount of inhibition is reduced at low light intensities and is greatest at light saturation, where the dark reaction determines the rate.

TABLE 7.

Concentration	At 700 lux			At 22,000 lux		
	Control	Treated	Percentage of control	Control	Treated	Percentage of control
KCN						
0.10 M	0.43	0.40	93	1.97	1.55	79
0.20 M	0.43	0.33	78	1.97	0.90	46
Phenylurethane						
0.006 M	0.68	0.49	72	1.69	1.48	87
0.007 M	0.56	0.37	67	1.90	1.50	79

Reduction of water content, therefore, retards the dark reaction and has little or no effect on the light reaction.

Treboux (1903), Pantanelli (1903), Bernbeck (1924), and Dastur (1925) observed a decline in photosynthetic rate with a loss of water and lowered cell turgidity. The retardation of the dark reaction observed in the present experiments with highly concentrated sucrose solutions perhaps indicates that water is involved in the dark reaction; but the possibilities are very complicated, and limitation of water content may serve to increase the concentration of inhibitors within the cell which are capable of affecting the dark reaction, or it may modify colloidal conditions of the cell on which the dark reaction depends.

In order to compare the results obtained by the pretreatment method employed in the present studies with the results obtained by Warburg during treatment, tests were made with solutions of potassium cyanide and phenylurethane. The rates (c.mm. O₂ per min. per 35 million cells) obtained at two light intensities are shown in table 7.

With potassium cyanide treatment, greater inhibition was obtained at high light intensity than at low, indicating a retardation of the dark reaction. Treatment with phenylurethane caused greater inhibition at low light intensity than at high, showing retardation of the photochemical reaction. These observations are in agreement with the results and conclusions of Warburg, and indicate, therefore, the suitability of the pretreatment method employed in these studies.

Little is known concerning the various ways in which the different inhibitors act. The effect of narcotics, like phenylurethane, is generally considered to be dependent upon their adsorption by interfaces in the cell, and their retardation of the photochemical reaction has been interpreted as due to an interference with the energy transfer from the light absorbent to another part of the photosynthetic mechanism (Franck and Gaffron, 1941). Inorganic substances, like CoSO₄ and (NH₄)₂SO₄, which strongly depress the light reaction, and others, such as KI, CuSO₄ and H₃BO₃, which retard it to a lesser extent, may also serve in various ways to bring about a hindrance of energy transfer. It is possible that some of these substances may inactivate the chloro-

phyll, although no visible color changes were observed. Thus, the effect of copper may have been brought about by the formation of copper chlorophyll. The peculiar sensitivity of algae to copper has never been adequately explained. HgCl₂ was observed to destroy chlorophyll and probably halted photosynthesis by this means.

Some of the observed inhibitions of the light reaction may be due to effects on dark reactions which may precede it, such as the absorption of carbon dioxide or the formation of a chlorophyllous carbon dioxide compound investigated by McAlister (1939). In essence this would mean that the actual light reaction would be unaffected by the inhibitor, except for the retardation resulting from interference with the prior dark reaction on which it is dependent. Adequate discussion of these relations is difficult at present, because it is not definitely known whether most or all of the reactions constituting the dark stage precede or follow those of the photochemical stage.

Restriction of the dark stage of the photosynthetic process may be brought about by interference with enzyme systems. Cyanides are known to be specific poisons for organometallic catalysts and are considered to affect the dark reaction by acting on its enzymes. Some of the inhibitors herein found to affect the dark reaction may act in a similar manner. The various salts studied probably affect different specific reactions of the dark stage rather than all reactions involved in this stage, as would be expected of temperature. Since salts of the heavy metals combine with proteins, retardation of the dark chemical stage, the photochemical stage, or both may have been brought about through a combination with the enzymes or other proteins involved. The findings of Emerson and Green (1938), that pH over a wide range had no effect on photosynthesis of *Chlorella*, seem to eliminate this as an influential factor in the inhibition observed in the present investigations. It is perhaps worthy of special emphasis that, although several of the inorganic compounds tested inhibited the dark stage alone and others retarded both dark and photochemical stages in various degrees, none was found which inhibited only the photochemical stage.

The results of the present survey, showing that several additional substances of diverse chemical na-

ture are able to affect the two principal sets of reactions in the photosynthetic process in ways similar to those of cyanides and urethanes, may be of value in further research on photosynthesis. A study of these substances in various combinations may provide other useful evidence by revealing synergisms and antagonisms. Although in their gross effects on the photochemical and dark reactions of photosynthesis the compounds studied constitute a few groups, it is expected that further analysis will show that they differ markedly in their individual mechanisms of action.

SUMMARY

The inhibitory effects of inorganic compounds on photosynthesis in *Chlorella vulgaris* were studied. Cells were suspended in test solutions for about thirty minutes; they were then rinsed with water, and their rates of photosynthesis in a carbonate-bicarbonate buffer were determined by means of Warburg manometers.

In tests at high light intensity very low concentrations of CuSO_4 , CoSO_4 , and HgCl_2 inhibited photosynthesis, but stronger solutions of ZnSO_4 , H_3BO_3 , $(\text{NH}_4)_2\text{SO}_4$, NiSO_4 , KCl , and KI were required to retard the process. The amount of inhibition tended to vary over a rather wide range of

concentrations, but HgCl_2 showed a narrow range between non-effective and completely inhibiting concentrations. The rates of respiration and of subsequent culture growth were not appreciably affected by treatment with concentrations which reduced photosynthesis 50 per cent. MgSO_4 , MnSO_4 , and KNO_3 inhibited photosynthesis only at concentrations wherein the high osmotic pressure of the solutions lowered the rate.

By means of studies at five different light intensities only the dark reaction was found to be retarded by ZnSO_4 , NiSO_4 , and KCl . The effect of CuSO_4 , H_3BO_3 , and KI was mostly a retardation of the dark reaction and a lesser inhibition of the light stage. CoSO_4 and $(\text{NH}_4)_2\text{SO}_4$ depressed both the dark and photochemical stages of photosynthesis about equally. No substance was found which inhibited only the photochemical stage. Decrease in water content, brought about by suspension of cells in sucrose solutions of high osmotic pressure, caused a retardation of the dark reaction only.

Some possible modes of action of these inhibitors are discussed.

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STIMULATORY AND TOXIC EFFECTS OF COPPER SPRAYS ON POWDERY MILDEWS¹

C. E. Yarwood

WHILE COPPER is toxic to many fungi at concentrations above 0.01 per cent and is the most widely used of all chemicals for the control of plant diseases, different fungi vary greatly in their sensitivity to it. At concentrations below 0.001 per cent copper is frequently stimulatory, and Steinberg (1939) regards copper as an essential element for several fungi. The literature on the stimulatory effects of copper at low concentrations for fungi which are grown in culture solutions and on the toxic effect of copper on plant parasitic fungi in general is very extensive and cannot be reviewed here.

The writer is not aware of detailed studies of the copper tolerance of powdery mildews, but copper fungicides are successfully used for the control of certain powdery mildews, though sulphur fungicides are generally considered superior (Martin, 1940). The writer (unpublished data) has secured control of several powdery mildews with several copper as well as several sulphur fungicides used as eradicator or protective treatments in field and greenhouse tests. Copper fungicides are especially useful for the control of powdery mildews on such sulphur-sensitive crops as cucurbits. The stimulation of powdery mildews by dosages of copper which are highly toxic to certain other plant disease fungi and even to powdery mildews under certain conditions is, therefore, of interest.

MATERIALS AND METHODS.—A strain of *Erysiphe polygoni* DC. from clover, *Trifolium pratense* L., and another from bean, *Phaseolus vulgaris* L. var. Pinto, were maintained on greenhouse plants. Bean plants were inoculated with the fungus or treated with the test fungicide, or both, when the primary, true, unifoliate leaves had reached about two-thirds full size. Inoculation was accomplished by dusting dry spores or spraying a water suspension of spores on the upper and lower surfaces of the leaves. Infection on the primary leaves was recorded within ten days after inoculation. Because of the heavy infection in the controls in these tests, counting of individual colonies was impractical and fungus development was rated on a relative scale of 0 to 10 in which 0 indicated no mildew mycelium apparent to the unaided eye, and 10 indicated that the leaf surface was entirely covered with mycelium and conidia. In germination tests 100 spores were counted for each treatment for each test.

Bordeaux mixture containing equal parts of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and CaO in water was prepared by adding 13 per cent hydrated lime to the specified amount of copper sulphate which had first been diluted with water to approximately spray strength, and the concentration of bordeaux is expressed as the percentage by weight of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in the final spray. The cottonseed oil used was a brand of self-

emulsifying oil containing approximately 85 per cent cottonseed oil and 15 per cent of a glyceryl phthallic ester (SEC oil of Röhm and Haas Company, Philadelphia). The cuproside dust contained 10 per cent red copper oxide, 10 per cent ferric oxide, and 80 per cent bentonite and diatomaceous earth (No. 10 copper dust of A. L. Castle, Mountain View). Spraying was done with a De Vilbiss atomizer using 35 pounds air pressure.

In studies of sprays as protective fungicides the plants were first sprayed with the test fungicide, and the film was allowed to dry for several hours before the plants were inoculated. In studies of sprays as eradicator fungicides the plants were first inoculated and the powdery mildew fungus was allowed to develop for several days before the test fungicide was applied. Because of their exposed mycelium most powdery mildews are uniquely adapted to comparative studies of the same chemicals as eradicator and protective fungicides, and powdery mildews have been extensively used in the evaluation of eradicator sprays (Martin and Salmon, 1932).

Different degrees of relative humidity were obtained by means of known concentrations of sulphuric acid in closed chambers, as described by Stevens (1916).

The term stimulation will be used in the broad sense to indicate the response of the organism in those treated units where the growth was greater than in the control units. The basic cause of the increased growth due to copper sprays is not understood. The term toxic is used in the usual sense to indicate that a given treatment reduced the growth of the organism as compared with the control.

RESULTS.—*Effect of copper on the germination of conidia.*—Conidia of clover powdery mildew dusted on the surface of 7 cc. of test solutions in syracuse watch glasses and held for ten hours in diffuse daylight gave the following germination values (average of five tests): water, 42 per cent; 0.1 per cent copper sulphate, 46 per cent; 1 per cent copper sulphate, 31 per cent; 0.1 per cent mercuric chloride, 31 per cent; and 1 per cent mercuric chloride, 4 per cent. These concentrations of copper and mercury are much higher than are tolerated by most parasitic fungi, and the writer believes that the high tolerance of *Erysiphe polygoni* to water solutions of copper and mercury may be related to the ability of the conidia of *Erysiphe polygoni* to germinate without an external supply of water (Yarwood, 1936b).

The presence of moisture may have an important bearing on the stimulatory and toxic action of copper for powdery mildews. The results of five tests averaged in table 1 show that on glass slides the dried deposit from 0.1 per cent bordeaux + 0.1 per cent cottonseed oil was highly toxic when water was added to the slides along with the clover powdery

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TABLE 1. *Effect of copper sprays on the germination of conidia of Erysiphe polygoni from clover.*

Test spray which was applied to slides and dried	Slides atomized with water, in moist chamber at 100 per cent relative humidity	Dry slides in moist chamber at 100 per cent relative humidity	Dry slides at 90 per cent relative humidity
	per cent	per cent	per cent
Water (control)	31	22	4.7
0.1 per cent bordeaux	30	24	5.8
0.1 per cent bordeaux + 0.1 per cent cottonseed oil	9.3	24	14.1
0.5 per cent cuprocide dust	0	0	0

mildew conidia and the slides incubated at 100 per cent relative humidity. On the other hand, this same deposit was highly stimulatory when the dry slides with dry spores were incubated at 90 per cent relative humidity. Results at 100 per cent relative humidity, but in the absence of free moisture, were intermediate. Similar results were secured in four tests with *Erysiphe polygoni* from bean, but the toxic action on the atomized slides and the stimulatory action on dry slides at 90 per cent relative humidity were less marked than with clover mildew. Similar dosages of bordeaux without cottonseed oil were only slightly stimulatory if at all, and similar dosages of copper in the form of cuprocide dust were toxic in all environments. All three forms of copper were toxic in all environments to barley powdery mildew (*Erysiphe graminis* DC.) in three tests.

Copper sulphate and bordeaux mixture at various dosages were suspended in plain agar containing 2 per cent sucrose and the poured plates of this substrate were dusted with the conidia of bean powdery mildew. In one representative test the percentage germination on sucrose agar containing 0.06 per cent bordeaux was 71 as compared to 50 in the control

plates, and the average length of the germ tubes in the bordeaux plates was 63 microns as compared to 58 in the control plates. In tests in which the germination in the controls was poor the increase of percentage germination and germ tube length due to copper was usually greater.

Effect of bordeaux spray on the development of bean mildew on leaves.—It has been observed on many occasions that, during periods of high light intensity, powdery mildew develops poorly or not at all on the upper surface of inoculated primary unifoliate bean leaves in the greenhouse, though it develops fairly well on the upper surface of secondary trifoliate leaves and excellently on the lower surface of the primary leaves. The nature of this inhibition by light is not understood, but shading the inoculated plants under such conditions usually favors mildew development on the upper surface of primary leaves. Dilute bordeaux mixture also stimulates mildew development under such conditions, and the results of one test illustrating what the writer believes to be the effect of light and dilute bordeaux on mildew development on the upper surface of primary bean leaves are given in table 2. In this test the pro-

TABLE 2. *Effect of light intensity and bordeaux spray on development of bean mildew on upper leaf surface.*

Spray treatment and incubation period	Outdoors, R.L.I. ^a 100	Clear greenhouse, R.L.I. 64	Half whitewashed greenhouse, R.L.I. 33	Whitewashed greenhouse, R.L.I. 18
	Mildew development	Mildew development	Mildew development	Mildew development
No spray (control):				
4 days	0	0	0	0
7 days	0	0.3	4	6
9 days	0	0.3	7	8
0.05 per cent bordeaux:				
4 days	0	0	3	2
7 days	0	3	9	10
9 days	0.3	7	10	10
0.1 per cent bordeaux:				
4 days	0	0	3	2
7 days	0	0.6	10	10
9 days	0	1.7	10	10

^a R.L.I. = Relative light intensity.

fective bordeaux sprays were applied at 2 p.m., August 6, 1941; at 4 p.m. the dry leaves were dusted with dry spores, and the inoculated plants were placed in the test environments. There was considerable alternation between clear weather and high fog during the nine days of this test, and the light intensity was measured with a Weston photometer on five occasions in each environment during the nine-day period. The outdoors reading for each time of observation was considered as 100, and the other values were expressed in relation to this, the average values being given in table 2. The environments chosen for this test were the outdoors and three different greenhouses with various amounts of shading in the form of a whitewash coating. In this test the light intensity in even the darkest environment was somewhat inhibitory to mildew development. It is realized that light was not the only important environmental factor which varied between these four conditions of exposure, but the writer believes it was the principal one responsible for the differences obtained. The amount of mildew for each treatment was estimated at four, seven, and nine days after inoculation. The results, presented in table 2, indicate that dilute bordeaux mixture stimulated the development of bean mildew under each light condition tested.

In the test just reported the plants were inoculated with dry spores and placed immediately in a dry test environment. When similar bordeaux-sprayed plants were inoculated by spraying them with spores in a water suspension and the inoculated plants were held overnight in a moist chamber before being placed on the greenhouse bench, the effect of the bordeaux spray was frequently, but not always, to reduce the mildew development on the sprayed plants to less than that on the control plants. Here, as with spores on slides (table 1), bordeaux mixture was apparently more toxic in the presence of free water than in its absence.

When dilute bordeaux was used as an eradicant spray, it usually reduced the powdery mildew development on the sprayed plants. In table 3 are given results of one representative test of bordeaux as a protective and as an eradicant spray for bean mildew. In such tests it is difficult to choose comparable conditions of treatment for the protective and eradicant sprays, since the conditions chosen are arbitrary

but may markedly influence the results. The importance of moisture as influencing the effects of protective sprays on powdery mildews has been discussed in the previous paragraph. In eradicant treatments, applications of bordeaux mixture and other fungicides were more effective in killing powdery mildew mycelium if the sprayed plants were kept wet for several hours after spraying than if the sprayed plants were dried immediately. It is known that water alone, sprayed at high pressures, is effective in eradicating powdery mildews (Yarwood, 1939a), but this possibility was guarded against in these tests by using low impact pressures in atomizing the plants.

In the tests reported in table 3, one set of plants was inoculated on February 5. At 10 a.m. February 11 these plants and a second set of uninoculated plants of the same age were sprayed with the different fungicides. At 4 p.m. February 11 the second set of plants was inoculated by dusting the plants with conidia. The first set of plants thus served as a test of the eradicant properties of the fungicides and the second set of plants as a test of the same materials as protective fungicides. Only the results with 0.1 per cent bordeaux and 0.1 per cent cottonseed oil are presented in table 3. In this test, which is representative of several tests of this type, leaves treated with 0.1 per cent bordeaux as a protective spray showed more mildew on their upper surfaces than the control leaves, while the same treatment as an eradicant spray killed practically all the mildew on the upper and lower leaf surfaces. Cottonseed oil and bordeaux plus cottonseed oil were also apparently more effective as eradicant sprays than as protective sprays.

The difference between the relative mildew on the upper leaf surface of the controls for the protective and eradicant applications (table 3) is not due to treatment, but is presumably due to differences in the light environment following the February 5 inoculation (for eradication) and the February 11 inoculation (for protection). Such differences have been quite common in successive inoculations. It is only when the environment has been inhibitory to mildew development on the upper leaf surface that copper stimulation of mildew on growing plants has been demonstrated.

On growing plants as well as on slides (table 1) there are important differences between different

TABLE 3. Comparison of eradicant and protective applications of bordeaux spray on the development of bean powdery mildew.

Spray	Protective application		Eradicant application	
	Relative mildew on upper leaf surface	Relative mildew on lower leaf surface	Relative mildew on upper leaf surface	Relative mildew on lower leaf surface
Water (control)	0	10	7	10
0.1 per cent bordeaux	5	10	0.5	0
0.1 per cent cottonseed oil	0	2.5	1	0.5
0.1 per cent bordeaux + 0.1 per cent cottonseed oil	0	0.5	0	0

forms of copper. No stimulation of powdery mildew has been observed from applications of cuprous oxide, either as pure cuproside or as cuproside dust. Also the cuproside dust has been uniformly more effective as a protective fungicide for powdery mildews than either cuproside or bordeaux when all were compared at the same copper content.

DISCUSSION.—It is commonly believed that non-gaseous fungicides must go into solution in order to be effective in killing fungi. Since most fungus spores apparently require free moisture for their germination, it has been difficult to test the truth of this assumption. However, the conidia of many powdery mildews do not require free moisture for their germination (Yarwood, 1936b) and are, therefore, adapted to such a study.

Tests reported here show that free moisture is not necessary for either the toxic or stimulatory action of copper fungicides for powdery mildews, though the bordeaux mixture used was more toxic in the presence of free water than in its absence. It therefore appears possible that the contact exchange theory of Jenny and Overstreet (1939), as applied to the absorption of nutrient ions by the roots of higher plants, might be applied to the absorption of copper by fungi and might explain how this can occur in the absence of water.

The results presented in this paper might be interpreted to indicate that copper as a plant nutrient was deficient for the powdery mildews studied under the conditions of these experiments. However, many other unusual treatments such as the addition of iron sulphate, potassium permanganate, potassium nitrate, and egg yolk to the culture medium, the treatment of the spores with heat, the use of spores at certain times of the day, and placing the dry spores on the dry leaf surface, may, under certain conditions, also be stimulatory to the conidia of certain powdery mildews (Yarwood, 1936a, 1939b, 1941).

The results presented are principally for *Ery-*

siphe polygoni from bean and clover. Similar tests with *Erysiphe graminis* from barley and with *Erysiphe cichoracearum* DC. from cucumber and cantaloupe have as yet shown no stimulation, but only toxicity, from copper fungicides.

This study tends to emphasize the importance of experimental conditions in studies of fungicides, since it is shown that a given dose of copper may be toxic, neutral, or stimulatory to the powdery mildews studied, depending on environment and other conditions of the tests. A related observation is that of Abe (1930), who found that 0.0004 to 0.006 per cent copper sulphate was stimulatory to *Piricularia oryzae* Br. et Cav. at 28°C. but was not at higher or lower temperatures.

SUMMARY

The conidia of *Erysiphe polygoni* from clover germinated well on the surface of 1 per cent copper sulphate. The dried deposit of 0.1 per cent bordeaux + 0.1 per cent cottonseed oil on glass slides was toxic to conidia of *Erysiphe polygoni* from red clover and bean in water, approximately neutral on dry slides at 100 per cent relative humidity, and stimulatory on dry slides at 90 per cent relative humidity. Copper sulphate or bordeaux added to sucrose agar increased the percentage germination and the length of the germ tubes of bean powdery mildew conidia. When light was inhibitory following inoculation, bean plants sprayed with 0.1 per cent bordeaux frequently showed more mildew development on the upper surface of primary leaves than did unsprayed plants. Bordeaux was more effective as an eradicant spray than as a protective spray for bean mildew, and no mildew stimulation has been observed when bordeaux was used as an eradicant spray.

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TRANSPORT OF THIAMIN IN THE TOMATO PLANT¹

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It is known that an external supply of thiamin is essential for growth of the isolated tomato root (Robbins and Bartley, 1937; White, 1937), and it may, therefore, be suspected that in the intact plant also thiamin is in some fashion associated with growth. The present paper deals with the production and distribution of thiamin in the tomato plant.

TECHNICAL.—The experiments to be discussed deal with girdled tomato plants. Seeds ("San Jose Canner" of the California Packing Corporation) were germinated in washed river sand having particles between 10 and 20 mesh. When the seedlings were three weeks old they were transferred to similar sand contained in four-inch clay pots. Hoagland's nutrient solution was supplied daily. These plants were used for experiments when they were between five and six weeks old, at which time they were eight to ten inches tall. In two types of experiments use was made of larger plants. These were grown in coarse gravel contained in two-gallon glazed crocks. All plants were greenhouse grown.

Girdling of the stems or petioles was done with a jet of steam directed through a glass nozzle having an orifice 1 mm. in diameter. This treatment appeared to kill, locally, all living elements of the stem. Within a few hours of the treatment the stem collapsed over a distance of approximately 1 cm. Leaves apical to the girdle did not wilt during a period of at least six days.

All plants were dried in a current of air at 60°C., weighed, ground, and assayed for thiamin content by the *Phycomyces* assay (Schopfer and Jung, 1937). Five \pm 0.1 mg. of plant material were weighed into each of a series of 50 cc. Erlenmeyer flasks. Ten cc.

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of thiamin-free basal medium² was added to each flask, and the flasks were then fitted with cotton plugs, autoclaved for fifteen minutes at 15 lbs. pressure, and inoculated with 0.2 cc. of a suspension of *Phycomyces Blakesleeanus* spores obtained from a standard stock culture. The cultures were incubated for six days at 25°C. in diffuse light, the mycelia removed, washed, dried and weighed. Controls with no added thiamin and with 0.02, 0.04, or 0.08 γ (micrograms) thiamin per flask were run in each experiment. The apparent thiamin per sample was computed on the basis of the controls with supplements of known amounts of thiamin. Table 1 gives the results of an experiment in which dried ground tomato leaf was assayed at three different levels of sample size. The apparent thiamin per sample is a linear function of the sample size for 2.5, 5.0 and 10.0 mg. samples within the accuracy of the determinations. The calculated thiamin per gm. of leaf tissue is hence independent of the sample size within the same limits of error. Table 1 also shows that added thiamin is quantitatively recovered in the presence of 2.5 or 5 mg. samples of dried tomato leaves. The leaves do not then appear to contain substances which interfere with thiamin determination by the *Phycomyces* method. Estimation of the thiamin content of the same material by the thiochrome method gave a mean value of 5.78 γ /gm.³ in close agreement with the mean value of 5.71 γ /gm. obtained in the *Phycomyces* assay.

Design of experiment.—With two exceptions (tables 10 and 11) the experiments discussed below were planned as follows. The plants for the experi-

² 0.5 gm. $MgSO_4 \cdot 7H_2O$, 1.5 gms. KH_2PO_4 , 20 gms. dextrose, 4 gms. recrystallized asparagin, and 1 cc. of microelement stock solution per liter of Pyrex redistilled water. The microelement stock solution contained 50 mg. $Fe_2(SO_4)_3$, 100 mg. $ZnSO_4$, 20 mg. $CuSO_4$, 10 mg. $MnSO_4$, 10 mg. H_3BO_3 and 20 mg. $(NH_4)_2MoO_4$ per liter.

³ Thiochrome assays by Sidney Gottlieb.

TABLE 1. Apparent thiamin content of tomato leaves assayed by *Phycomyces* at three different sample size levels and in the presence and absence of added thiamin. Five replicate determinations in each case.

Sample	2.5 mgs. dried tomato leaves	5.0 mgs. dried tomato leaves	10.0 mgs. dried tomato leaves	2.5 mgs. dried tomato leaves + 0.04 γ thiamin	5.0 mgs. dried tomato leaves + 0.04 γ thiamin
Calculated thiamin γ /sample	0.0155	0.0300	0.053	0.0520	0.0695
	0.0120	0.0300	0.056	0.0555	0.0735
	0.0145	0.0285	0.062	0.0545	0.0665
	0.0135	0.0300	0.056	0.0570	0.0775
	0.0145	0.0285	0.056	0.0530	0.0665
	0.0140 \pm 0.0006	0.0294 \pm 0.0004	0.0566 \pm 0.0015	0.0544 \pm 0.0009	0.0707 \pm 0.0021
Thiamin γ /gm.	5.60 \pm 0.24	5.88 \pm 0.08	5.66 \pm 0.15
Thiamin recovered	0.0404	0.0413
Per cent thiamin recovered	101	103

ment were selected for uniformity from about 1.5 times the needed number. The selected plants were then randomized in groups of eight and each treatment of the experiment was carried out on one such group. Each group was harvested as four samples of two plants each. Each sample of two plants was composited, dried, and analyzed in duplicate. The degree of variability in such an arrangement was tested in a uniformity trial with untreated material and table 2 shows the results obtained with respect to dry weight of tops and thiamin assays. The maximum difference in top dry weight between replicate groups of eight plants amounted to 10.5 per cent of the average dry weight per group, while the average difference from the mean was 3.3 per cent. The results of a variance analysis of the assay data of table 2 are given in table 3. It should be noted that the variance of samples (item c of table 3) is significant when tested against the variance associated with duplicate assays (item d of table 3). Therefore, the variation due to sampling is larger than the analytical error in the experiment.

The standard error of a group mean of four samples (each sample consisting of the mean of two assays) is 0.564 as derived from the 16 mean values of table 2, and the standard error of a difference between two such group means would be 0.80. The difference necessary for significance (between two group means) would be 1.70 at the 5 per cent level of probability (15 degrees of freedom) and 2.36 at the 1 per cent level, the latter value representing a difference between the two means of about 10.6 per cent. This is, however, only a minimum value for the difference between two means necessary for significance in an actual experiment since the variability of plants subjected to varying treatments was larger than the variability of untreated plants, as those of table 2. In general, however, a difference between two means of 25–30 per cent or more was found to be significant as can be seen in tables 5, 6, 7, and 8.

DISTRIBUTION OF THIAMIN.—Table 4 shows the concentration of thiamin in various parts of a series of tomato plants 40 cm. tall. Analyses were done on the apical growing point (including leaves less than approximately 4 cm. long), on each leaf, on the stem, and on the roots. There was a marked concentration gradient of thiamin in these tomato plants in the sense that the highest concentration was found in the apex and successively lower concentrations in each successively older leaf. A similar gradient would appear to occur in the stem. Table 4 also gives the calculated total amount of thiamin per leaf or other part. This figure is the product of the dry weight of the part by its thiamin concentration. The amount of thiamin per leaf was small in the young leaves, rose to a maximum at the 8th leaf, and fell to a small amount in the oldest leaf. This distribution of thiamin in the tomato plant, which has been confirmed in four further experiments on a total of sixty-four plants, does not of itself offer any clue as to the precise site of formation of the substance, a question

which formed the subject of the following experiments.

ACCUMULATION OF THIAMIN ABOVE A GIRDLED PORTION OF STEM.—Nine groups of eight plants each were drawn randomly from a selected set of seventy-two plants. One group was harvested at once. The

TABLE 2. Total top dry weights and *Phycomyces* assay results on four groups of eight plants each, drawn randomly from 32 selected tomato plants averaging approximately eight inches tall. *Phycomyces* assays on 5 mg. samples. Controls with known amounts of thiamin gave: 0.00 γ = 0.0 mgs., 0.02 γ = 10.75 mgs., and 0.04 γ = 22.5 mgs. of *Phycomyces mycelium*.

	Dry wt. gms./2 pl.	Mgs. <i>Phyco- myces</i> rep- licates	Means
Group I:			
Sample 1 (2 pl.)....	0.50	22 23	22.5
Sample 2 (2 pl.)....	0.53	22 22.5 23.5	22.25
Sample 3 (2 pl.)....	0.51	23.5 23.5	23.5
Sample 4 (2 pl.)....	0.57	22 20.5	21.25
Average	0.53 \pm .015		22.4 \pm .46
Group II:			
Sample 1 (2 pl.)....	0.58	22 20	21.0
Sample 2 (2 pl.)....	0.58	23 23	23.0
Sample 3 (2 pl.)....	0.66	20 22	21.0
Sample 4 (2 pl.)....	0.55	20 20.5	20.25
Average	0.59 \pm .024		21.3 \pm .59
Group III:			
Sample 1 (2 pl.)....	0.61	23 24	23.5
Sample 2 (2 pl.)....	0.55	23 22	22.5
Sample 3 (2 pl.)....	0.55	20 21	20.5
Sample 4 (2 pl.)....	0.61	22 22	22.0
Average	0.58 \pm .017		22.1 \pm .62
Group IV:			
Sample 1 (2 pl.)....	0.60	23.5 24.5	24.0
Sample 2 (2 pl.)....	0.47	23 20.5	21.75
Sample 3 (2 pl.)....	0.59	22 20.5	21.25
Sample 4 (2 pl.)....	0.62	22 23.5	22.75
Average	0.57 \pm .034		22.4 \pm .61

TABLE 3. *Analysis of variance of Phycomyces data from uniformity experiment reported in table 2.*

Source of variation	Dg. F.	Sums of squares	Mean squares	F.
Total	31	51.375		
Between groups of 4 samples (hypothetical treatments)	3	6.437	(b) 2.146	1.23 ^a
Between lots of 2 (between samples)	12	31.688	(c) 2.641	
Within lots of 2 (between duplicate assays)	16	13.250	(d) 0.828	3.19 ^b

^a Not significant.^b Significant at 5 per cent level of probability.

plants of four further groups were girdled at the second node by the application of a jet of steam. The remaining four groups served as further controls. Twenty-four hours later, one group of girdled plants and one group of control plants were harvested, and this operation was repeated on the second, fourth, and sixth days after girdling. Each control plant was separated into top (above the second node), roots (below the sand level) and stem sample (1 cm. of stem at the second nodes). Each girdled plant was separated into top, root, stem (1 cm.) immediately above the girdle, and stem (1 cm.) immediately below the girdle. The results of the dry weight determinations and thiamin assays on the top and root samples are given in table 5. The total thiamin contained in the tops and in the roots of the control plants increased markedly during the six days of the experiment. In the tops of the girdled plants, total thiamin also increased, although less markedly than in the control plants. In the roots of the girdled plants, however, total thiamin did not increase significantly beyond the amount initially present and, in fact, decreased markedly after the second day.

Table 6 gives the results of dry weight determinations and thiamin assays on the stem sections from the experiment of table 5. It is evident that there was a marked accumulation of thiamin above the girdle and some depletion of thiamin from below the girdle. The accumulation was apparent one day after girdling, and, on a concentration basis, reached its final maximum by the second day. On the basis of the total thiamin per cm. of stem, however (and associated with the swelling of the stem above the girdle), the accumulation continued during the entire six days of the experiment. On the sixth day, the concentration of thiamin in the 1 cm. of stem above the girdle was 2.8 times as great as the concentration in the corresponding portion of stem in the control plants and 3.5 times as high as the concentration in 1 cm. of stem below the girdle. The total amount of thiamin in 1 cm. of stem above the girdle was 4.8 times as great as the amount in the corresponding portion of control stem and 12.2 times as great as the amount in 1 cm. of stem below the girdle. The results of this experiment, which were confirmed in four similar experiments, suggest that thiamin may be normally transported in living elements of the stem.

ACCUMULATION OF THIAMIN BELOW A GIRDLED PORTION OF STEM.—In this experiment stems of tomato plants were girdled between the mature leaves and the young rapidly growing leaves. Each plant was arbitrarily divided into an apex, possessing leaves less than approximately 3 cm. long, and a portion with three mature leaves. Any leaves of intermediate size were removed. Each stem was marked midway between the lowermost young leaf and the uppermost mature leaf, and some of the plants were girdled at this point. At the time of harvesting each plant was separated into: (1) the apex (above the marker on the stem); (2) a 1 cm. portion of stem at the marked region, or in the case of girdled plants, portions of stem both above and below the girdle; (3) the three mature leaves; and (4) the roots. Table 7 gives data from an experiment in which six days were allowed to elapse between girdling and final harvesting. Only the results pertaining to the apex and the stem are given. Table 7 shows that the girdle interfered markedly with the growth of the young leaves, and that the total amount of thiamin above the girdle did not change greatly during the experiment, although the total thiamin content of the corresponding portion of the control plants increased threefold during the same time. The most striking feature of this experiment, however, was the marked accumulation of thiamin below the girdled portion of stem. The concentration of thiamin below the girdle was nearly twice the concentration above the girdle. The concentration of thiamin immediately above the girdle was slightly (but significantly) higher than that in the final control stem. This is probably not to be regarded as an accumulation, however, since the concentration above the girdle is insignificantly different from the concentration in the same portion of stem at the beginning of the experiment.

The accumulation of thiamin below an apical girdle, an observation which has been confirmed in four further experiments similar to that outlined above, suggests that in the plant there may be a movement of thiamin upward in the stem. The fact that thiamin is accumulated above a basal girdle but below an apical girdle further suggests that the mature leaves may export this substance.

TABLE 4. *Distribution of thiamin in tomato plants 40 cm. tall.*

Part	Wt. of part gms./2 pl.	γ thiamin per gm.	γ thiamin per part/2 pl.
Apex	0.113	19.8	2.24
Leaf 12.....	0.102	16.0	1.63
Leaf 11.....	0.172	14.8	2.54
Leaf 10.....	0.270	13.2	3.56
Leaf 9.....	0.356	11.4	4.06
Leaf 8.....	0.536	10.9	5.84
Leaf 7.....	0.516	9.4	4.86
Leaf 6.....	0.528	8.1	4.28
Leaf 5.....	0.392	7.8	3.06
Leaf 4.....	0.344	7.0	2.41
Leaf 3.....	0.242	5.7	1.38
Leaf 2.....	0.161	4.7	0.76
Leaf 1.....	0.137	4.5	0.62
Top 10 cm. of stem	0.310	10.0	3.10
Next cm. of stem..	0.620	4.2	2.60
Next cm. of stem..	0.572	3.0	1.72
Next cm. of stem..	0.662	2.4	1.59
Roots	1.135	6.7	7.61

EFFECT OF LEAF REMOVAL ON THE DISTRIBUTION OF THIAMIN.—The following experiment was designed to evaluate the relative importance of young and mature leaves in relation to the accumulation of thiamin above a basal girdle. Each of a series of plants was divided into an apex of young leaves and a portion possessing three mature leaves, as described in the preceding experiment. The growing points were then removed from one group of plants and the three mature leaves from a second group. Each group was next girdled at the second node. After six days the plants were harvested, dried, weighed, and assayed for thiamin. Table 8 gives the results pertaining to the regions of stem immediately above and below the girdle in the two groups. In the plants deprived of mature leaves there was no accumulation of thiamin above the girdle. In the plants with mature leaves but deprived of apices there was on the contrary a marked accumulation of thiamin above the girdle. This experiment, which was confirmed by two similar experiments, suggests that the presence of mature leaves is associated with thiamin accumulation in the stem of tomato plants.

Table 9 concerns an experiment similar to that outlined in table 8, except that the plants were not girdled. Removal of the mature leaves checked development of the apex and also diminished the total thiamin increase in the apex during the eight-day experimental period. It should be noted, however, that the apex did increase appreciably in total thiamin, indicating either some capacity for thiamin elaboration in the young leaves or, possibly, transport of thiamin stored in the stem to the growing point. The removal of the apex in the experiment of table 9 was associated with a marked increase in total thiamin content of the mature leaves, a phenomenon which is illustrated more strikingly in the following experiment.

EFFECT OF BUD REMOVAL ON THIAMIN DISTRIBUTION.—The plants used for this experiment were somewhat older than those used previously and were approximately twelve inches tall. All of the plants were decapitated and all but three leaves per plant were removed. One group of plants was allowed to develop new axillary shoots. All lateral buds, as they became evident, were removed from the other group.

TABLE 5. *Concentration of thiamin in various parts of tomato plants at various times after girdling of the stem at the second node.*

Treatment	Gms. dry wt.	γ B ₁ /gm.	γ B ₁ /2 pl.
0 days after girdling			
Tops:			
Control78±.031	7.07±.41	5.5
Girdled
Roots:			
Control12±.004	3.24±.34	.39
Girdled
2 days after girdling			
Tops:			
Control	1.03±.058	7.56±.20	7.8
Girdled80±.038	7.32±.20	5.9
Roots:			
Control19±.020	3.56±.08	.68
Girdled15±.007	2.72±.23	.41
4 days after girdling			
Tops:			
Control	1.35±.065	7.32±.05	9.9
Girdled	1.00±.041	7.12±.34	7.1
Roots:			
Control27±.012	3.21±.24	.87
Girdled097±.005	2.71±.07	.26
6 days after girdling			
Tops:			
Control	1.90±.030	7.52±.28	19.3
Girdled	1.19±.058	8.05±.35	9.6
Roots:			
Control32±.028	3.20±.28	1.02
Girdled085±.0065	2.53±.10	.21

After two weeks the plants were harvested, dried, and assayed. The results are given in table 10. Those plants from which buds were removed surpassed the plants upon which lateral buds grew out as to dry weight of the mature leaves, root dry weight, thiamin concentration of mature leaves and roots, and total thiamin content of mature leaves and roots. The accumulation of thiamin in the roots of the disbudded plants is particularly marked. This result, which has been confirmed in a second similar experiment, suggests that shoots and roots may compete for thiamin supplied by the mature leaves.

THIAMIN ACCUMULATION IN GIRDLED PETIOLES.—The following experiment indicates not only that mature leaves of the tomato may export thiamin but also supplies information relative to the vigor with which leaves of different ages carry on this process. Two tomato plants, approximately three feet tall and growing in gravel culture, were trimmed of lateral

TABLE 6. *Concentration of thiamin in the stems of tomato plants at various times after girdling at the second node.*

Days after girdling	Control			1 cm. above girdle			1 cm. below girdle		
	Dry wt. mgs./2 pl.	γ B ₁ /gm.	γ B ₁ /2 pl.	Dry wt. mgs./2 pl.	γ B ₁ /gm.	γ B ₁ /2 pl.	Dry wt. mgs./2 pl.	γ B ₁ /gm.	γ B ₁ /2 pl.
0	24	3.6 \pm 0.20	.087
1	24	3.5 \pm 0.20	.084	25.5	5.5 \pm .14	.14	21.5	3.0 \pm .16	.064
2	24	3.05 \pm 0.20	.073	28	8.1 \pm .21	.23	20	2.6 \pm .17	.052
4	31	2.85 \pm 0.11	.088	44	8.6 \pm .52	.38	20	2.7 \pm .07	.054
6	39	2.95 \pm 0.09	.115	67	8.2 \pm .72	.55	19	2.35 \pm .03	.045

TABLE 7. *Distribution of thiamin in tomato plants after girdling of the stem between the apex and the mature leaves.*

Days after girdling	Treatment	Apices		
		Dry weight mgs./2 pl.	γ B ₁ /gm.	γ B ₁ /2 pl.
0	control	65 \pm 4.3	13.2 \pm 0.67	0.86
6	control	304 \pm 19.6	8.4 \pm 0.36	2.55
6	girdled	101 \pm 8.8	9.3 \pm 0.59	0.94
1 cm. sections of stem in girdled region				
0	control	7.0 \pm 0.71	6.4 \pm 0.36	0.045
6	control	11.5 \pm 0.65	5.2 \pm 0.24	0.060
6	above girdle	7.8 \pm 0.48	7.0 \pm 0.26	0.055
6	below girdle	10.5 \pm 0.65	13.5 \pm 1.43	0.142

shoots. Senescent leaves at the base of the stem were also removed. The remaining leaves were next numbered consecutively starting at the base of the plant and petioles of alternate leaves girdled between the stem and the most proximal leaflets. After seven days, 1 cm. sections were harvested from the petiole of each control (not girdled) leaf and similar sections above and below the girdle were taken from the girdled leaves. Each petiole section was dried,

weighed, and assayed in duplicate for thiamin. The results of this experiment and of a second similar experiment are given in table 11. The younger control (not girdled) petioles had higher thiamin concentrations than the older petioles indicating a concentration gradient in the petioles similar to that obtaining in the entire leaves (table 4). No significant accumulation of thiamin was found on the stem side of any of the girdled petioles. Accumulation of thiamin was

TABLE 8. *Effect of leaf removal on the accumulation of thiamin above a basal girdle.*

Treatment:	Mature leaves removed, girdled at 2nd node		Mature leaves present, apex removed, girdled at 2nd node	
	Part:	1 cm. of stem above girdle	1 cm. of stem below girdle	1 cm. of stem above girdle 1 cm. of stem below girdle
Dry weight mgs./2 pl.....		22 \pm 0.95	19 \pm 0.85	42.5 \pm 1.32 20 \pm 1.55
γ B ₁ /gm.		5.4 \pm 0.20	5.4 \pm 0.43	13.6 \pm 0.94 4.8 \pm 0.55
γ B ₁ /2 pl.....		0.119	0.103	0.578 0.096

TABLE 9. *Influence of leaf removal on the distribution of thiamin in tomato plants.*

Part	Treatment	Dry wt. mgs./2 pl.		
		γ B ₁ /gm.	γ B ₁ /2 pl.	
Apex	initial control	30	16.7	0.50
	final control	191	15.8	3.02
	plants without mature leaves	104	14.7	1.53
Mature leaves	initial control	249	9.8	2.44
	final control	314	7.8	2.45
	plants without apex	365	11.0	4.02

TABLE 10. *Effect of bud removal on the distribution of thiamin in the tomato plant. The leaf data are based on duplicate assays on ten individual plants and the root data on five groups of two plants each.*

Part	Gms. dry wt./2 pl.		B ₁ /gm.		B ₁ /2 pl.	
	With buds	Without buds	With buds	Without buds	With buds	Without buds
Three mature leaves.....	1.02±.06	1.94±.13	5.96±.17	6.99±.18	6.08	13.6
Roots	0.51±.047	0.76±.045	5.7 ±.25	10.7 ±.52	2.91	8.13

TABLE 11. *Accumulation of thiamin in girdled petioles of tomato plants.*

No. of leaf from bottom	Treatment	Experiment #1			Experiment #2			Per cent increase in dry wt. of leaf in expt. # 1
		Normal	γ B ₁ /gm. Above girdle	Below girdle	Normal	γ B ₁ /gm. Above girdle	Below girdle	
1	girdled	..	13.4	3.5	..	18.1	2.7	..
2	normal	4.4	3.6	10
3	girdled	..	15.4	3.6	..	20.0	3.8	..
4	normal	4.3	4.0	12
5	girdled	..	13.7	3.4	..	21.6	3.5	..
6	normal	4.2	3.7	14
7	girdled	..	15.8	3.5	..	23.7	4.5	..
8	normal	4.5	6.3	23
9	girdled	..	13.9	4.0	..	23.6	5.2	..
10	normal	4.5	5.4	52
11	girdled	..	11.2	5.3	..	17.9	5.5	..
12	normal	5.1	4.9	91
13	girdled	..	7.7	4.4	..	12.9	5.0	..
14	normal	5.7	5.5	130
15	girdled	..	5.7	4.4	..	10.9	6.8	..
16	normal	7.5	5.5	550

found, however, on the laminar side of the girdled petioles. The accumulation was greatest in the oldest petioles and was smallest in the youngest girdled petiole. Owing to the fact that petioles at least 3 cm. long were needed for the experiment, it was not possible to girdle still younger leaves. In the experiment of table 7, however, the stem was girdled below these younger leaves, and there was no accumulation above the girdle.

The last column of table 11 gives the per cent increase in dry weight of the control (ungirdled leaves) during the seven days of experiment 1. Two leaflets from one side of each leaf were removed at the beginning and the two opposite leaflets were removed at the end of the experiment. The oldest leaves increased in dry weight by 10 to 23 per cent, whereas the youngest leaf increased by 550 per cent. Although the data are slight, still it can be said that accumulation of thiamin in the girdled petioles is in a general way complementary to the growth activity of the leaf in question.

DISCUSSION.—It has been shown that in the vegetative tomato plant there is a thiamin gradient, the highest concentration of this substance being found at the top of the plant in the youngest leaves, with successively lower concentrations in each successively older leaf. A similar gradient was found in the stem. The results of the experiments outlined above

do not suggest, however, that thiamin is transported from young leaves of high thiamin concentration to older leaves and roots having lower thiamin concentrations. Insofar as accumulation above a girdle can be taken to indicate transport in the normal plant, these experiments indicate that thiamin is produced in mature leaves and that this thiamin accumulates in other actively growing parts of the plant. Accumulation of thiamin would appear to be principally in portions of the plant toward which a transport of other materials such as carbohydrate would be expected.

SUMMARY

The distribution of thiamin in the tomato plant was investigated with the aid of an assay based on the thiamin requirement of *Phycomyces Blakeslee-anus*. Data are presented to show that substances interfering with the assay are not present in tomato leaves.

The highest concentration of thiamin in the vegetative plant was found in the youngest leaves. Each successively lower leaf contained a lower concentration of the substance.

Thiamin accumulated markedly above a girdle made by steaming the stems of tomato plants at the second node.

Thiamin accumulated markedly below a girdle made by steaming the stems of tomato plants in the region between the mature leaves and the young rapidly expanding leaves.

The roots of decapitated plants from which all axillary shoots were removed contained more thiamin than the roots of decapitated plants from which axillary shoots were allowed to grow.

Comparative thiamin accumulation in girdled petioles of leaves of different ages indicated that thiamin may be exported by mature leaves having relatively low concentrations of the material rather than by young leaves having higher concentrations of the substance.

STUDIES ON *CHLORELLA VULGARIS*. V. SOME PROPERTIES OF THE GROWTH-INHIBITOR FORMED BY *CHLORELLA* CELLS¹

Robertson Pratt

CELLS OF *Chlorella vulgaris* cultured in a synthetic medium under conditions that are highly favorable for growth produce a substance (or substances) that tends to retard their further multiplication (Pratt, 1940; Pratt and Fong, 1940a). It has been shown that this substance is water-soluble, and that it is capable of passing through the cell membranes, so that it becomes partitioned between the external solution and the *Chlorella* cell contents. The criticisms that have been directed against much of the similar work on protozoa, i.e., that depletion of nutrients, changes in hydrogen-ion concentration, and variable protozoan or bacterial populations in the culture medium influenced the results, have been ruled out of these studies on *Chlorella* (Pratt and Fong, 1940a). Later work by the same investigators has indicated that similar results might be expected whether the nitrogen that is supplied to the cells is combined in nitrate or ammonium ions (1940b).

The work that is reported below was undertaken in an attempt to ascertain some of the properties of the inhibiting agent or agents.

MATERIALS AND METHODS.—The cells were cultured as previously described in a solution that contained KNO_3 , 0.025 M.; KH_2PO_4 , 0.018 M.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 M.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.00001 M.; and potassium citrate, 0.00001 M. Microtrophic elements are apparently furnished in sufficient abundance as impurities in the ordinary C. P. grade of salts to satisfy the requirements of *Chlorella* (Pratt, 1941). Therefore, additional amounts were not added in the present experiments as they were in the earlier work. The initial pH value of this nutrient solution was 4.45. A gas mixture that was composed of 5 per cent CO_2 and 95 per cent air was bubbled continuously through the solutions, and the cultures were illuminated continuously from below by water-cooled Mazda lamps that yielded approximately 10,000 lux at the level of the flasks. The temperature varied from 18 to 22°C.

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Growth, as indicated by the number of cells in the cultures, was estimated from haemocytometer counts. Cells for inoculating new cultures in the different experiments were always withdrawn from vigorously growing four-day-old suspensions. They were washed by repeated centrifugalization and rinsing in distilled water. The initial population in all experiments was 100 cells per cu. mm., and the initial pH value, except as otherwise noted, was always adjusted to 4.45 ± 0.05 by the addition of KOH or H_2SO_4 . All cultures were shaken twice daily to insure uniform suspension of the cells.

The water used in these experiments was triple distilled, the second and third distillations being carried out entirely in Pyrex glass systems fitted with efficient spray traps. Distillation one was carried out in an ordinary laboratory still. The product that was obtained was treated with KMnO_4 and NaOH and was then distilled in an all Pyrex system. The third distillation, performed without any reagents, was likewise in an all Pyrex system. One set of stills was used exclusively for the second distillation and another set for the third distillation.

EXPERIMENTS AND RESULTS.—I. *Diffusibility of the inhibitor.*—The results of earlier work (Pratt and Fong, 1940a) showed that, as the age of *Chlorella vulgaris* cultures advances, the concentration of growth-inhibitor in the external solution increases, and it was suggested that in old cultures the high concentration in the external solution would tend to retard further outward diffusion of the inhibitor, thus resulting in its accumulation within the cells themselves. Experimental evidence was adduced to support the thesis that cells from old cultures are highly charged with the growth-inhibiting substance, and that, when they are transferred to fresh solutions that lack the inhibiting factor, this substance diffuses out into the surrounding medium.

Therefore, to obtain cells that were highly charged with the growth-inhibitor in the present investigation, cultures were prepared in the usual way and

were permitted to grow for 41 days, at the end of which time they contained approximately 99,000 cells per cu. mm. Most of the cells were removed from 2 liters of solution by centrifugalization and were rinsed twice by suspension in distilled water and recentrifugalization. Then these cells were suspended in 800 ml. of distilled water in a collodion sac that was in turn suspended in 8.5 liters of distilled water which was stirred frequently. The preparation was kept in darkness at 23°C. and periodically 75 ml. portions were withdrawn from the outer solution. The samples that were withdrawn were used to prepare new nutrient solutions with the same concentrations of salts as were used in the standard medium.

The results of two similar experiments performed at different times are shown in figure 1 in which the relative density of population in the different cultures after five days (compared with the population in standard stock cultures of the same age) is plotted on the ordinate, and the length of time the cells were in distilled water before the samples were withdrawn for preparing the nutrient solutions is plotted on the abscissa. Marked exosmosis of various cellular contents in addition to the inhibiting agent would be expected to occur under the conditions of this experiment. Similar subsequent tests with cells from young cultures (two to three days), however, failed to reveal any pronounced effect, either stimulatory or inhibitory, and it seems reasonable to assume, therefore, that the results plotted in figure 1 represent the effects of the *Chlorella* growth-inhibitor that is normally liberated by growing cultures and that they were not brought about by some other cell constituent.

It is apparent that the substance which is produced by *Chlorella* cells and which retards their growth is capable of passing through collodion membranes. The membrane that was employed prevented the passage of eosin and of methyl orange from 0.01 per cent solutions. Somewhat coarser membranes permitted the passage of these dyes. The possibility that electrical or other phenomena prevented passage through the finer membrane has not been definitely eliminated, but it seems safe to conclude tentatively that the molecules of the active substance are smaller than those of eosin which have been calculated to have a diameter of approximately 15 Å units.

The high point that occurs in each of the curves deserves some attention. It might be interpreted to mean that in *Chlorella*, as is true of the response of many organisms to other toxic substances, very low concentrations of the inhibitor are beneficially stimulating; or it might indicate that at least two substances or groups of substances, one stimulatory and the other inhibitory, are liberated by the cells. According to the latter idea, figure 1 would indicate that the accelerating substance is more readily diffusible than the inhibitor, since its influence on the external solution was manifest first, but that it is physiologically less active or is produced less abundantly or is more unstable than the inhibitor.

Although several interpretations of the data that are plotted in figure 1 are conceivable, the simplest one seems to be that the curves reflect in some measure the rate of diffusion of the inhibitor (which may be stimulating in low concentrations), and that they indicate that after 25 to 30 hours an equilibrium was established between the solutions within and without the collodion sac so that there was no further increase in the toxicity of the solutions for *Chlorella*.

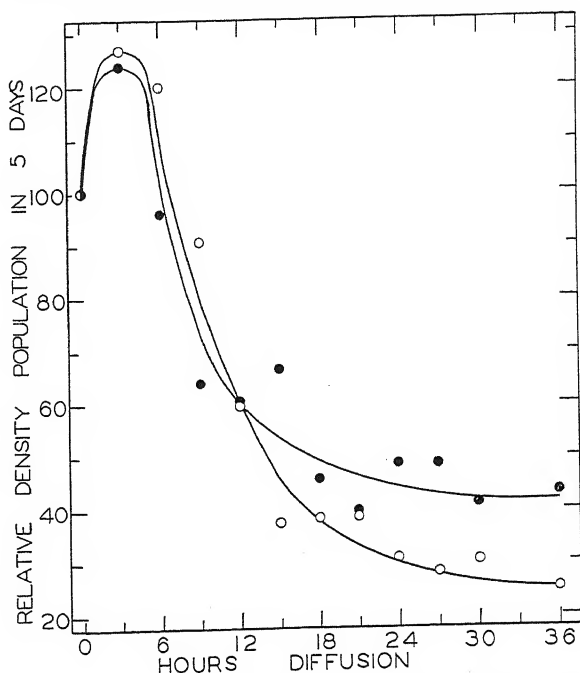


Fig. 1. Density of population in cultures of *Chlorella vulgaris* (expressed as percentage of that in control cultures prepared with distilled water) in solutions prepared with water in which *Chlorella* cells in a collodion membrane had been suspended for different periods of time.

II. *Solubility of the inhibitor.*—Cells were removed by centrifugalization from a 21-day-old culture that contained approximately 90,000 cells per cu. mm. and were dried for ten days over H_2SO_4 at a pressure of 3–5 mm. of Hg. Extractions were made in different solvents by placing 500 mg. of dried cells in 50 ml. of the particular solvent for twelve hours, decanting, and then adding 50 ml. of fresh solvent. After a second twelve-hour period the two portions of the solvent were combined and were evaporated to dryness without heat. Distilled water was added to the residue to redissolve the water-soluble fraction, thus eliminating the chlorophyll and much of the extraneous organic matter that was extracted from the cells by the different solvents. Then the preparation was filtered and nutrient solutions were prepared with this water, the volume being adjusted so that the extract from 500 mg. of dried cells was dissolved in 150 ml. of culture medium. These solutions were inoculated and cell counts were made periodically for about one week. The results of this experiment are

presented in figure 2 and table 1. They show that the inhibitor is soluble in the three solvents tested, i.e., 95 per cent ethyl alcohol, petroleum ether, and ether, and they suggest that it may be about twice

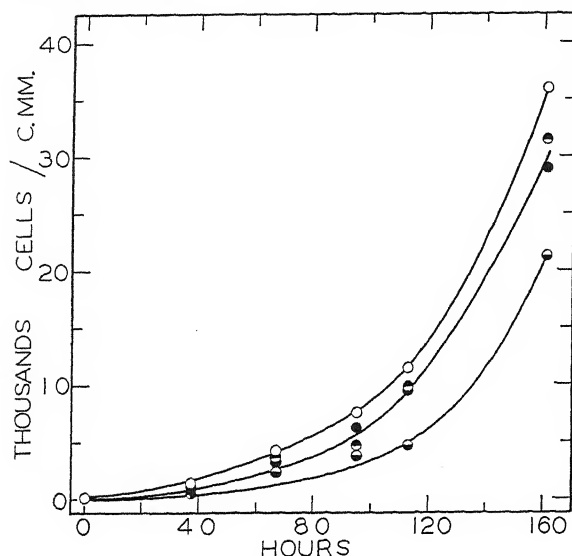


Fig. 2. Growth of *Chlorella vulgaris* in solutions containing material extracted in ether (circles with solid uppers), petroleum ether (solid circles), and 95 per cent ethyl alcohol (circles with solid lowers). The open circles represent growth in control cultures prepared with distilled water.

as soluble in the first of these as in either of the other two. The possibility should not be overlooked, however, that some other extraction technique would yield somewhat different values. It has been shown that the amount of growth hormone that is extractable from maize endosperms depends in large measure upon the extent of hydration of the tissue (Avery, Creighton, and Shalucha, 1940), and that in several types of plant tissues water is essential for

the extraction of auxin (Thimann and Skoog, 1940). Although the growth-retarding factor in *Chlorella* seems to differ in some respects from the recognized auxins, it is conceivable that some of the problems involved in its extraction may be similar to those encountered in plant hormone studies.

III. *Heat stability of the inhibitor.*—Cultures were prepared as in previous experiments and were permitted to grow for thirty days at the end of which time the mean density of population of the different cultures was 101,500 cells per cu. mm.

Two liters of the suspension were completely freed of cells by centrifugalization and subsequent passage of the supernatant liquid through Jena-glass filter sticks, No. 17G. The pH of the resultant filtrate was 6.55. These two liters were divided into ten portions of 200 ml. each. One portion was used immediately and without further treatment for the preparation of a new culture solution, the pH of which was adjusted to 4.5.

Each of the other portions was placed over a hot flame and rapidly brought to the boiling point, after which gentle boiling was maintained for different lengths of time. New culture media with pH values of 4.45 ± 0.05 were prepared from each of the different solutions after they were cool, and the original volume was restored by the addition of distilled water. All solutions were then inoculated and the density of population in the different cultures was determined after seven days.

The results that were obtained are plotted in figure 3 (open circles) where the density of population in the different cultures relative to that in the normal control solution is plotted on the ordinate, and the length of time the different solutions were boiled is shown on the abscissa. This experiment demonstrated clearly that the inhibitor is destroyed by boiling, and that the destruction is approximately a linear function of the length of the exposure to boiling. It did not show, however, whether or not oxidation

TABLE 1.

Relative growth of <i>Chlorella vulgaris</i> in solutions containing material extracted from <i>Chlorella</i> cells in:				
Time, hours	95% ethyl alcohol	Petroleum ether	Ether	Control: no extract
0	100 ^a	100 ^a	100 ^a	100
37	43.6 ^b	58.7 ^b	56.7 ^b	100
67	55.4	79.0	83.0	100
95	50.7	80.6	60.5	100
113	49.9	80.0	85.2	100
161	58.8	80.4	87.5	100
Averages	51.7	75.7	74.6	
	53.7*	80.0*	79.1*	

^a These figures are not included in the averages.

^b These figures are omitted from the averages marked with asterisks. It is apparent that these figures are considerably lower than the general trend in the respective series. It is not clear at present whether or not their omission can be justified on the grounds that an adaptation period was required for adjustment to the new environment in the different cultures and that the first observations are not as reliable as the later ones. Therefore, averages calculated both ways are given.

played a part in the destruction. It was repeated, therefore, with a modified technique that would tend to prevent oxidation or at least reduce it to a minimum.

Nitrogen that had been passed over hot copper and that had been cooled subsequently by passing through coils submerged in ice water was bubbled through the solutions for twelve hours before they were heated and also while they were boiling. The results are plotted in figure 3 (solid circles). Although the relative populations in the cultures of this experiment differed appreciably from those in the previous one, the general character of the results is similar. After the initial periods, in the results for which some deviation might be expected, since a short period of time was required to elevate the temperature of the solutions to the boiling point, both curves are approximately linear and both have nearly the same slope. The fact that the growth that is represented by the second curve (solid circles) was consistently 10 to 15 per cent less than that in the first experiment (indicating higher concentrations of inhibitor in the solutions) may mean that the destruction of the inhibitor in the first experiment was due in part to oxidation. However, it seems clear that an oxida-

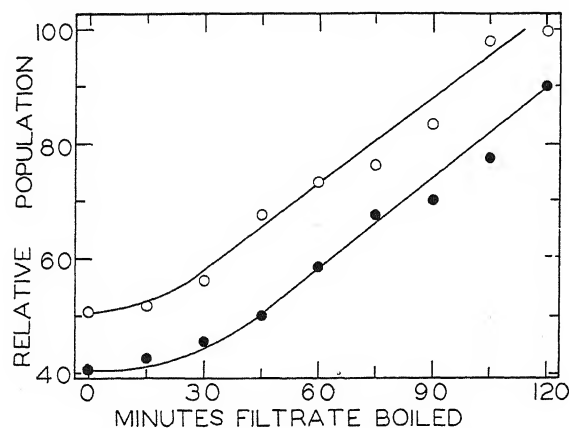


Fig. 3. Density of population in cultures of *Chlorella vulgaris* (expressed as percentage of that in control cultures prepared with distilled water) in solutions prepared with *Chlorella* filtrate that was boiled for different periods of time. Open and solid circles represent different experiments. See text for explanation.

tive mechanism could have accounted for only a small part of the inactivation, and that destruction was probably accomplished principally by hydrolysis or pyrolysis, or perhaps by both.

In another experiment 500 mg. of dried *Chlorella* cells were heated at 100°C. for two hours in a continuous stream of nitrogen that had been passed over hot copper and that had then been cooled. These cells were extracted with 95 per cent ethyl alcohol as described above. The extract was evaporated to dryness and the water-soluble fraction was taken up in distilled water as outlined earlier. This extract was found to be incapable of significantly inhibiting the

growth of *Chlorella*. Although this experiment does not eliminate hydrolysis as a possible mechanism for effecting destruction of the *Chlorella* inhibitor, it does demonstrate that such destruction may be brought about by heat alone.

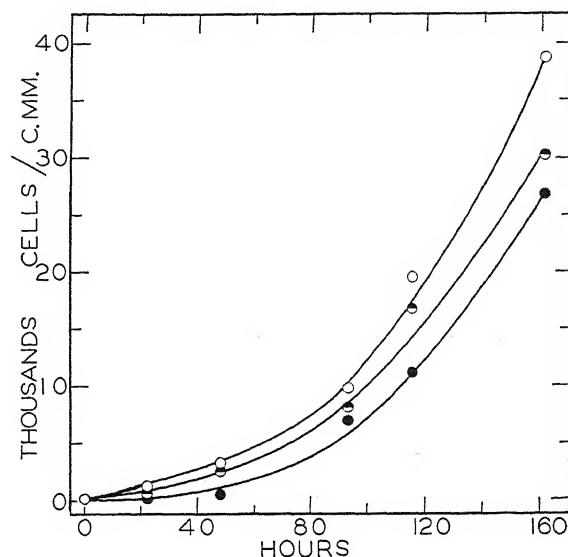


Fig. 4. Growth of *Chlorella vulgaris* in solutions containing material extracted by ether from aqueous *Chlorella* filtrate at pH 4.1 (circles with solid uppers), and pH 8.8 (solid circles). The open circles represent growth in control cultures prepared with distilled water.

IV. *Extraction of the inhibitor from acid and alkaline solutions.*—Cells were removed from a 28-day-old culture by the usual centrifugalization and filtration. The pH of the resulting filtrate was 6.3. Two 200 ml. portions were withdrawn, one of which was acidified to pH 4.1 and the other alkalized to pH 8.8 by the addition of H_2SO_4 and KOH , respectively. Each portion was extracted with dry ether for eighteen hours, after which time the ether extracts were removed and were evaporated to dryness without heat. Each extract was suspended in 200 ml. of distilled water which was stirred for two to three hours. Then the samples were filtered, and the filtrate was employed in the preparation of new cultures. Cell counts were made periodically during approximately one week.

The data from this experiment are shown in figure 4 and table 2. The curves show the actual cell counts in each of the cultures at different times, and the tabularized data show the growth in the two experimental cultures relative to that in the control culture prepared in the usual way with distilled water.

Clearly, the inhibitor is more easily taken up in ether from an alkaline aqueous solution than from one with an acid reaction. This may be interpreted to mean that the inhibiting substance is an organic base which is converted into a salt in an acid medium. Sufficient quantities of the compound to permit chemical analysis have not yet been separated.

TABLE 2.

Time, hours	Relative growth of <i>Chlorella vulgaris</i> in solutions containing material extracted by ether from aqueous <i>Chlorella</i> filtrate adjusted to:		
	pH 4.1	pH 8.8	Control: no extract
0	100 ^a	100 ^a	100
22	61.5 ^b	11.2 ^b	100
48	82.2	17.5 ^b	100
93	87.8	74.0	100
115	86.1	60.5	100
161.5	78.3	69.3	100
Averages	79.2	46.5	
	83.6*	67.9*	

^a These figures are not included in the averages.

^b These figures are omitted from the averages marked with asterisks. It is apparent that these figures are considerably lower than the general trend in the respective series. It is not clear at present whether or not their omission can be justified on the grounds that an adaptation period was required for adjustment to the new environment in the different cultures and that the first observations are not as reliable as the later ones. Therefore, averages calculated both ways are given.

V. Activity of the inhibitor in acid and basic media.

—Accepting the assumption that the *Chlorella* inhibitor is a basic compound, it seemed of interest to determine whether its retarding action on growth is greater in the basic form, which might or might not be dissociated, or in the salt form, which would be expected to be at least partially dissociated in aqueous solutions. One observation that may bear on this point is the fact that the growth of *Chlorella* in solutions with the salt composition used in these experiments is less vigorous in media with initial pH values of about 6.0 than in those with initial values of about 5.20 or less (Pratt and Fong, 1940b).

The problem is complicated, however, because, under the conditions of the experiments, the hydrogen-ion concentration changes continuously (Pratt and Fong, 1940b). Furthermore, as might be expected, and as these workers showed, the hydrogen-ion concentration of the medium is so intimately related to other factors concerned in the growth of *Chlorella* that changes in the pH value immediately bring about a readjustment among numerous other component forces that influence the rate and amount of growth.

Nonetheless, several culture solutions were prepared with the cell-free filtrates obtained from 32-day-old cultures, the average population of which was 98,300 cells per cu. mm. The initial hydrogen-ion concentrations were adjusted to different levels so that the pH values ranged from 3.5 to 7.2. No precipitation was evident in any of the cultures even at the higher pH values. All media were clear at the time of inoculation.

The data that were obtained are presented in figure 5 where ordinates represent the relative density of population in the different cultures after seven

days (compared with the population in the control cultures prepared with distilled water) and the abscissas show the initial pH values of the different solutions. The solid and open circles represent data from two separate experiments that were performed at different times.

Although the quantitative agreement of the data from the two experiments is poor and even the apparent shapes of the curves seem to be somewhat different, one significant feature is evident in both curves, viz., the left extremity is markedly higher than the right. As the initial hydrogen-ion concentration decreased (increase in pH) the amount of growth, relative to the control, also decreased.² The inhibitor was more effective in checking growth at relatively high pH values than at lower ones.

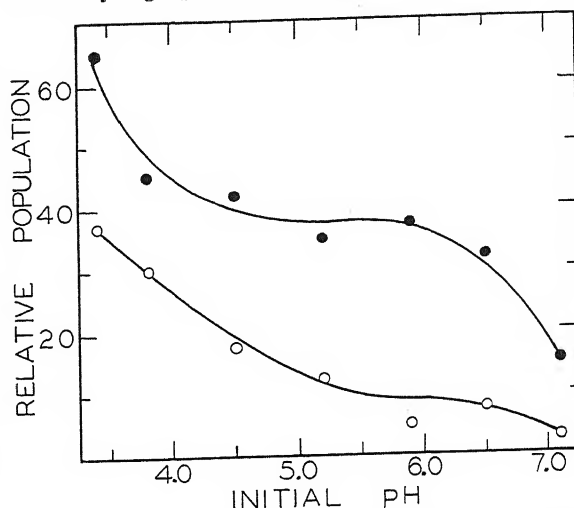


Fig. 5. Density of population in cultures of *Chlorella vulgaris* (expressed as percentage of that in control cultures prepared with distilled water) in solutions prepared with *Chlorella* filtrate and adjusted to different initial hydrogen-ion concentrations. Open and solid circles represent two different experiments.

As was pointed out before, this type of experiment is beset by many complications, and it would be dangerous to make a categorical interpretation of the results. The data presented in figure 5 do not prove that the inhibitor is more effective in the basic form, but since the value of the ratio base/salt should increase with the pH, they may be considered as preliminary circumstantial evidence that this is so.

VI. Do *Chlorella* cells produce a substance that is different from the inhibitor and that accelerates their growth?—It was pointed out above that the curves in figure 1 might indicate the presence of two substances, one that accelerates growth and another that inhibits growth, in the diffusate from the *Chlorella* suspension. This is a point on which it is difficult to give a definite answer at present. Positive results from experiments might prove the existence of two

² Growth was poor in all solutions, including the controls, with pH values of 6.0 or higher, but since the results are all relative, valid comparisons may be made.

substances but negative results would not necessarily disprove their existence. Several experiments were performed in an attempt to solve the problem.

It seemed that if a specific accelerator were formed, and if it diffused through collodion membranes into distilled water more readily than the inhibitor, one might expect the solution retained by the membrane to contain a relatively higher effective concentration of inhibitor after a short time than a similar solution that had not been so separated from distilled water. The net inhibiting action of such a solution should be greater, since some of the accelerator that would tend to nullify the effect of the inhibitor would have been removed.

Accordingly, such an experiment was prepared. Cells were treated as described in section I, and one portion of the dense cell suspension was placed in a collodion bag that was suspended in distilled water, while another portion was retained for a control. After three hours and again after six hours, cells were removed from 200 ml. portions of the suspension within the collodion sac and from the original suspension that had not been dialyzed. Fresh culture media were prepared from the cell-free solutions and they were inoculated with new cells. The experiment was repeated three times but in no case were significant differences in the growth in the different cultures detected.

It seemed possible that if two substances that exerted opposite actions were present, they might differ in some of their properties, such as solubility, heat lability or stability, etc.

Accordingly, cell-free samples removed from within the collodion sac after three and six hours, respectively, were compared with similar cell-free samples removed from the control suspension.

In one set of experiments the filtrates were heated following treatment with nitrogen, as described in section III and their effect on the growth of *Chlorella* was tested. No significant differences were detected, indicating that if two substances of opposing action are formed by *Chlorella* cells, both are destroyed by heat at the same rate.

Similar negative results were obtained from experiments that were designed to test the relative solubilities of the two substances, if such existed, in ether, petroleum ether, and 95 per cent ethyl alcohol.

DISCUSSION.—The object of the experiments that are reported in this paper was to ascertain some of the properties of the growth-retarding substance that is formed by *Chlorella* cells. A discussion of the literature that deals with the evidence for and against the production of auto-inhibitive and auto-stimulative substances by microorganisms would, therefore, be out of place. It does seem worth while, however, to point out that, despite the vast literature on the subject, there are relatively few clear cut cases in which the production of auto-inhibitive substances has been conclusively demonstrated. Virtual proof of the existence of such compounds among micro-organisms

has been published by Mast and Pace (1938; protozoa), Pratt (1940; *Chlorella*), Pratt and Fong (1940a; *Chlorella*), and Kidder (1941; protozoa). Similar observations have been recorded for echinoderms (Springer, 1922; Peebles, 1929).

None of the attempts to determine the nature of the retarding agents from the different organisms has been particularly fruitful, principally because the compounds are formed in such minute quantities that attempts to isolate appreciable amounts of them have been uniformly unsuccessful. It seems possible that these naturally-formed growth-inhibiting agents may prove to be among the most potent of biologically-produced chemicals. Mast and Pace (1938) estimated that the substance formed by *Chilomonas paramecium* exerted a measurable effect when present in the culture solution in a concentration of 1 part in 800,000,000.

The inhibitor formed by *Chlorella* cells resembles that found in the protozoa, *Chilomonas paramecium* (Mast and Pace, 1938), and *Tetrahymena geleii* (Kidder, 1941) in that it is destroyed by heat.

Peebles (1929) and Kidder (1941) have published evidence that seems to indicate that during the course of development growth-accelerating substances may be produced along with growth-inhibiting agents. It was pointed out that some of the data in the present report could be interpreted as indicating the presence of a growth-accelerator in the *Chlorella* cultures. Subsequent experiments that were designed specifically to elucidate this question failed to yield positive experimental evidence to support such an idea, however, and the simpler hypothesis for the present seems to be that the increased growth that was observed under some conditions was merely the result of stimulation from exposure to exceedingly low concentrations of a "poison."

SUMMARY

The experiments that are reported in the present paper constitute a continuation of previous studies on the growth of the uni-cellular green alga, *Chlorella vulgaris*, in which it was shown that during the course of their development *Chlorella* cells produce a growth-inhibiting substance. Experiments that were designed to reveal some of the properties of the growth-retarding agent are described.

It was found that the inhibitor diffuses through collodion membranes, and that its molecules are probably less than 15 Å units in diameter.

The inhibitor is soluble in 95 per cent ethyl alcohol, ether and petroleum ether, being about twice as soluble in the first of these solvents as in either of the other two.

It is destroyed by heat. Relatively little, if any, of the destruction is caused by oxidation.

The inhibitor is more readily extracted by ether from an alkaline aqueous solution than from one with an acid reaction. From this it is concluded that the active agent is probably an organic base.

Chlorella filtrate that was highly charged with growth-inhibitor checked growth more effectively at relatively high pH values (6.5 to 7.0) than at relatively low values (3.5 to 4.0). This indicates that it is more effective in the basic form than in the salt form that might be expected to exist in the more acid media.

Exceedingly low concentrations of the inhibitor seem to exert a beneficially stimulating effect on *Chlorella* cells.

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SOME RESPONSES OF VALENCIA ORANGE SEEDLINGS TO VARYING CONCENTRATIONS OF CHLORIDE AND HYDROGEN IONS¹

H. E. Hayward and Winifred M. Blair

MUCH of the citrus produced in the western and southwestern states is grown under conditions involving some degree of salinity of the soil solution. It has seemed desirable to extend the investigations noted below to include the anatomical and physiological responses of citrus seedlings to controlled concentrations of chloride and hydrogen ions. Since the root is in most intimate contact with the soil solution, emphasis has been placed on the structural and functional behavior of that organ in relation to the conditions imposed upon the experimental plants.

The nutritional requirements of citrus seedlings have been investigated with respect to nitrogen and the absorption of other essential ions (Breazeale, 1919; Chapman and Liebig, 1937, 1940); and the response of seedlings grown in water cultures to various environmental factors—temperature, aeration, and hydrogen ion concentration—has been studied (Girton, 1927). The absorption of chloride by rough lemon and *Poncirus trifoliata* seedlings in relation to the intake of nitrate, calcium, and sodium ions has been investigated by Haas and Reed (1926), and the same authors have reported on the effects of sodium, potassium and calcium on young orange trees (1923). The anatomy of citrus roots has been examined in relation to osmotic pressure and periodicity

of growth (Cossmann, 1939), and the developmental anatomy of the seedling and roots of the Valencia orange has been described by Hayward and Long (in press).

METHODS AND EXPERIMENTAL DESIGN.—The seedlings were grown in water culture to permit periodic observation of the developing roots and to determine their condition with respect to lateral root formation, production of root hairs and other growth responses. Since it has been noted (Hoagland and Arnon, 1938) that aeration of the culture solution may have a marked effect on the development of the plant, half of the cultures under each treatment were aerated by a low pressure continuous flow system while the other half were maintained without aeration.

Selected Valencia orange seeds were germinated in *Sphagnum* moss in September, 1940, and in November 288 of the most uniform were divided into thirty-six groups of eight each. Each lot was assigned by chance to one of the designated cultures which were randomized on greenhouse benches.

Three culture solutions were used: (1) the base nutrient solution, Hoagland's number two (1938),² (2) the base nutrient solution plus 50 m.e. chloride per liter, (3) the base nutrient solution plus 100 m.e.

² This solution contains the following salts:

Salts	m.e./l.
$\text{NH}_4\text{H}_2\text{PO}_4$	3
KNO_3	6
$\text{Ca}(\text{NO}_3)_2$	8
MgSO_4	4

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TABLE 1. *Number of leaves per plant and percentage of chlorosis and tip burn.*

Series	Salt treatments	No. of plants	Leaves per plant	Chlorosis, ^a per cent	Tip burn, ^b per cent
A (pH 5.5-6.0, unaerated)	1—B. N.	25	8	0	1
	2—50 m.e. Cl ⁻ /l.	23 ^c	7	1	7
	3—100 m.e. Cl ⁻ /l.	18	6.3	23	16
AO (pH 5.5-6.0, aerated)	1—B. N.	24	7.4	0	0
	2—50 m.e. Cl ⁻ /l.	22	6.6	10	7
	3—100 m.e. Cl ⁻ /l.	11	5.0	58	38
B (pH 7.5-8.0, unaerated)	1—B. N.	24	4.8	3	0
	2—50 m.e. Cl ⁻ /l.	21	5.3	26	22
	3—100 m.e. Cl ⁻ /l.	10	4.0	62	50
BO (pH 7.5-8.0, aerated)	1—B. N.	24	6.8	12	1
	2—50 m.e. Cl ⁻ /l.	22	5.1	40	22
	3—100 m.e. Cl ⁻ /l.	11	3.5	73	71

^a Based on average severity of symptoms for three replications.^b Percentage of leaves showing symptoms.^c See mortality figures, table 4.

chloride per liter. The chloride for solutions 2 and 3 was supplied as NaCl 50 per cent, MgCl₂ 25 per cent and CaCl₂ 25 per cent. Micro-elements were added in the following concentrations: boron, 0.5 ppm.; manganese, 0.5 ppm.; zinc 0.05 ppm.; copper 0.02 ppm.; molybdenum, 0.01 ppm. Five ppm. of iron was added initially as citrate, and further additions were made as needed.

Two levels of hydrogen ion concentration were maintained by daily adjustment of the pH values

with HNO₃ or KOH: Series A—pH value within range 5.5 to 6.0; Series B—pH value within range 7.5 to 8.0. Half of the cultures in each series (designated with an O) were aerated with a carbon pipe aerator operated under a continuous pressure of approximately 8 pounds. The other half (without the O designation) were unaerated. Each treatment was replicated three times.

All the seedlings were started in the base nutrient solution, and the pH value was adjusted within the

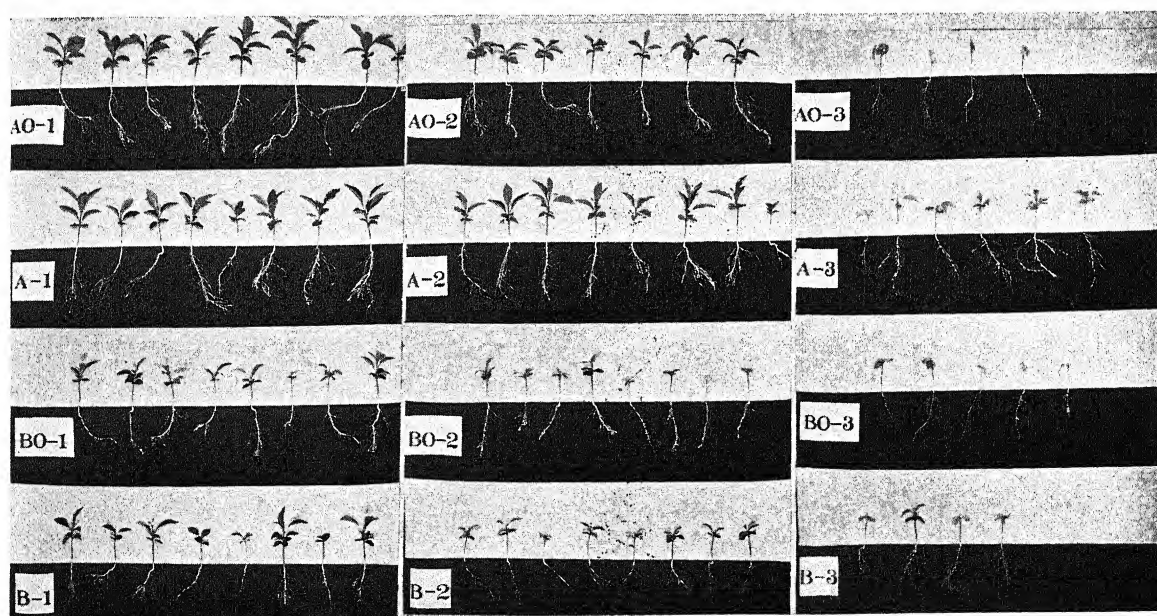


Fig. 1. Valencia orange seedlings at the conclusion of the experimental period showing relative development under the various treatments. Each panel represents the seedlings in one replication. The A and AO series were maintained at pH 5.5-6.0, the B and BO series at pH 7.5-8.0. The cultures designated with an O were aerated. No. 1 cultures contained the base nutrient solution, No. 2 the base nutrient solution with 50 m.e. chloride per liter added, No. 3 with 100 m.e. chloride per liter added. The smaller number of plants in the No. 3 cultures represents the approximate mortality incurred under that treatment.

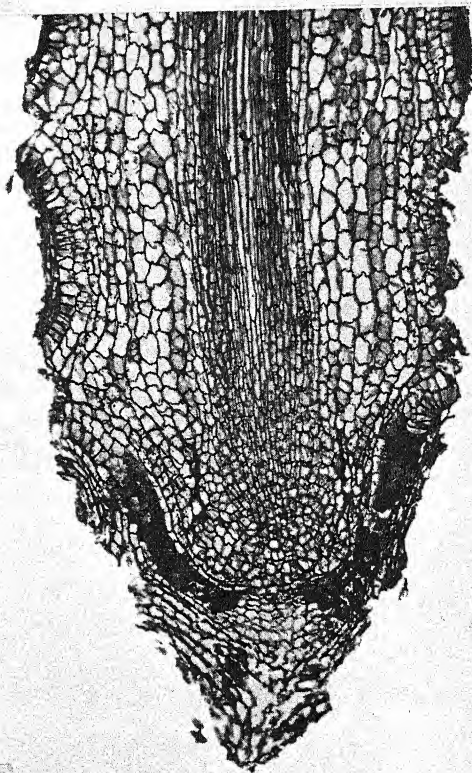
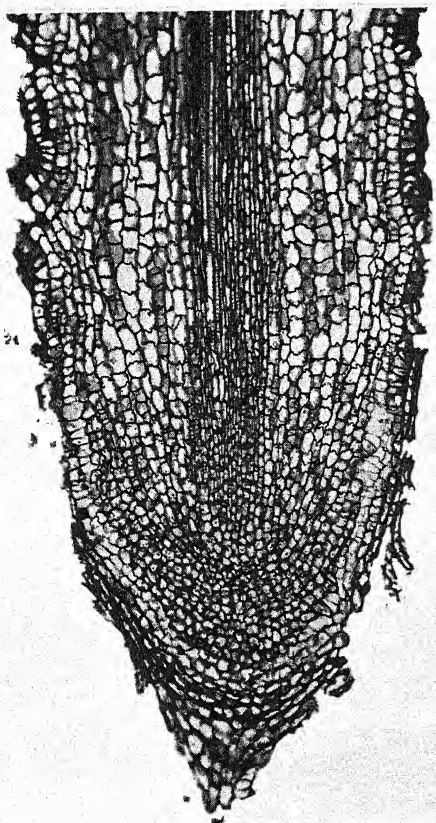
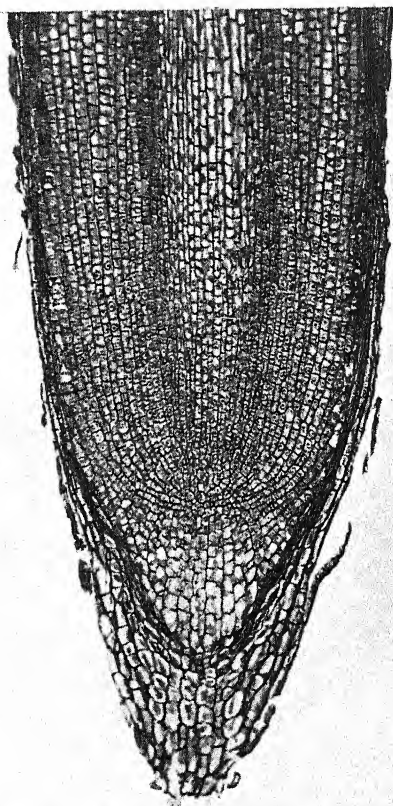
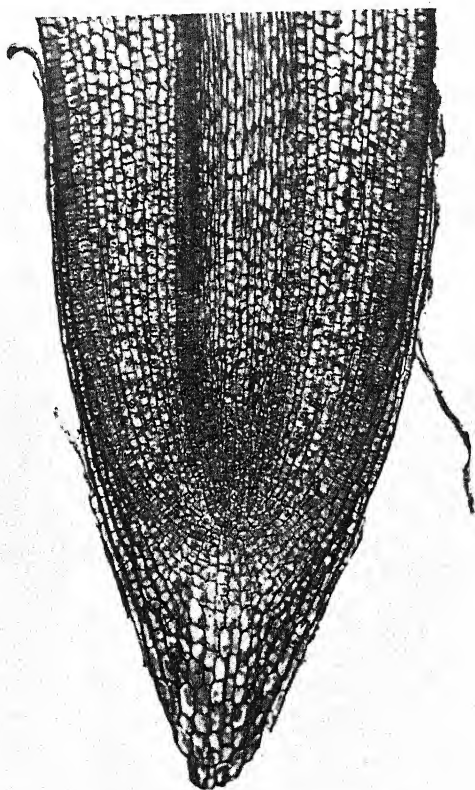


TABLE 2. *Harvest data. Summary.*

Series	Salt treatments	No. of plants	Total length cm.	Average per plant					
				Fresh wt. mg.	Dry wt. mg.	Fresh wt. roots mg.	Dry wt. roots mg.	No. lateral roots	No. white root tips
A (pH 5.5-6.0, unaerated)	1—B. N.	25	22.7	1,750	360	520	90	20.8	8.8
	2—50 m.e. Cl ⁻ /l.	23	22.4	1,580	290	420	70	17.5	6.4
	3—100 m.e. Cl ⁻ /l.	18	19.9	1,130	180	320	50	15.8	9.6
AO (pH 5.5-6.0, aerated)	1—B. N.	24	22.9	1,730	340	550	100	23.0	10.5
	2—50 m.e. Cl ⁻ /l.	22	20.3	1,310	230	370	60	17.5	5.5
	3—100 m.e. Cl ⁻ /l.	11	18.0	750	130	260	40	13.4	2.2
B (pH 7.5-8.0, unaerated)	1—B. N.	24	18.0	940	170	360	60	11.2	6.7
	2—50 m.e. Cl ⁻ /l.	21	17.6	840	130	300	40	14.9	.6
	3—100 m.e. Cl ⁻ /l.	10	17.3	640	100	240	30	14.4	.3
BO (pH 7.5-8.0, aerated)	1—B. N.	24	20.6	1,220	230	440	80	20.9	2.0
	2—50 m.e. Cl ⁻ /l.	22	18.8	910	140	310	60	16.7	.5
	3—100 m.e. Cl ⁻ /l.	11	16.8	500	100	210	30	12.9	.1

specified range of the series. In treatments 2 and 3, the chloride salts were added by increments of 25 milliequivalents per liter at two-day intervals until full concentration was reached, and complete changes of all solutions were made as required. The plants were grown for about two months, the final harvest being January 28, 1941.

GROSS MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES.—Differences in leaf color were detectable between the A and B Series within a week after the solutions were up to full concentration; and, at the end of two weeks, the leaves of plants grown at the lower pH value were definitely darker green. Chlorosis was more pronounced at the high chloride levels in all series, but was intensified by high pH values (table 1). Under equivalent salt treatments, the plants in aerated cultures were somewhat more chlorotic.

Chloride injury was indicated by tip burn of the leaves. This symptom was noted first in the high chloride cultures two weeks after the solutions were brought to full concentration. The degree of burning was conditioned by the hydrogen ion concentration of the solution and by aeration at the highest chloride level. Burning was most severe in the aerated cultures of the 100 m.e. chloride solution maintained at pH 7.5-8.0 and least in base nutrient solutions regardless of aeration (table 1). The tip burn was progressive and tended to involve more and more of the blade until the entire leaf became brown and desiccated. This was most pronounced in the high salt-high pH cultures.

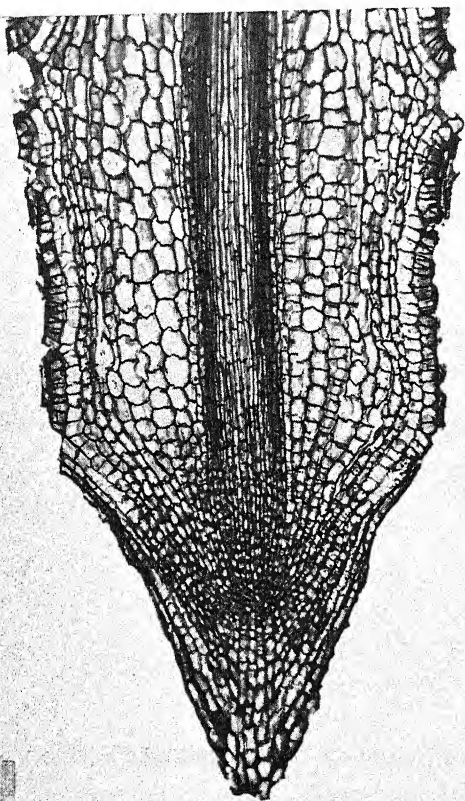
Growth was inhibited at the high chloride levels, and this was intensified by low hydrogen ion concentration. There were fewer, smaller leaves and fewer, shorter, lateral roots on plants grown under these conditions (fig. 1). A highly significant reduction

in the total length of plants, total fresh and dry weights, number of lateral roots and white root tips occurred under the high chloride treatments. The effect of the higher pH value was also highly significant; in fact, the response to this factor was greater than to increased chloride treatment. On the basis of all treatments, no significance could be determined for aeration, although, at the lower pH value, aeration did influence the dry weight of seedlings significantly. The failure to get significant differences between aerated and non-aerated cultures might have been due to the presence of enough oxygen in the non-aerated solutions to meet the requirements of the slowly growing roots. The harvest data are shown in table 2.

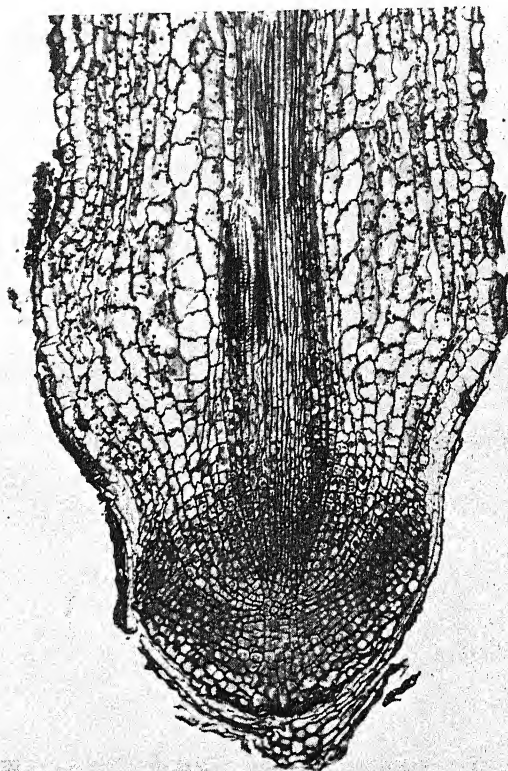
ROOT DEVELOPMENT.—The response of a plant to varying conditions of the soil or nutrient solution is intimately associated with the anatomy and physiology of its roots. Numerous investigators have observed that the extent of the absorbing zone of a root will vary with changes in external conditions, such as soil moisture, temperature, and aeration. Scott (1928) refers to the effect of temperature on the water roots of *Salix*, and Plaut (1910, 1919) reports the closure of the absorbing zone during conditions unsuitable for growth in forest trees. The development of a protective suberized layer, the metacutis, which closes the absorbing zone has been described for a number of plants by Kroemer (1903-4), Plaut (1919), and Müller (1906). Cossmann (1939) has pointed out that suberization in the root of citrus belongs to a simple type where a metacutis to two to three rows of cells is formed in the root cap adjacent to the growing point.

The roots of citrus grow slowly and may exhibit alternating phases of activity and dormancy (Reed and MacDougal, 1937). During the active period,

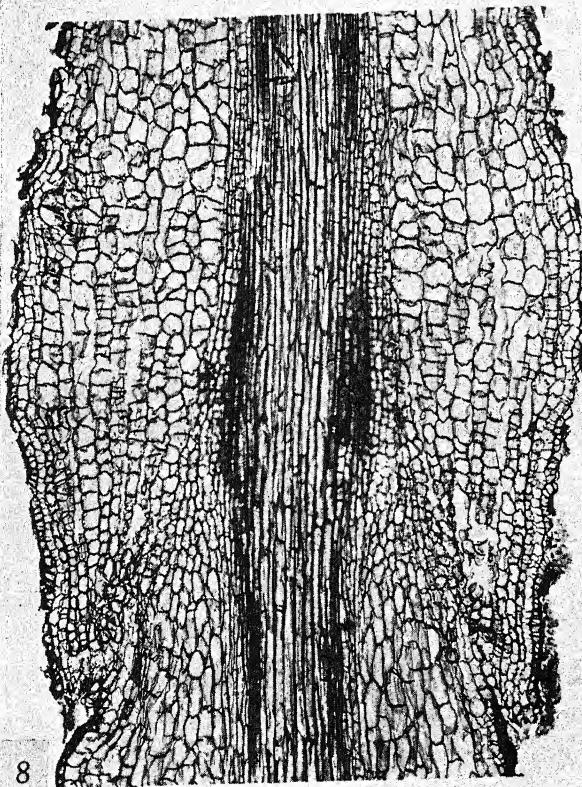
Fig. 2-5.—Fig. 2. Median longitudinal section of an active root tip showing meristematic region and root cap.—Fig. 3. The same, showing the initiation of the metacutis, indicated by the zone of deeply stained cells extending through the root cap.—Fig. 4 and 5. Later stages in growth inhibition showing roots in the dormant state with heavily cutinized epidermis and metacutis and reduced root cap.



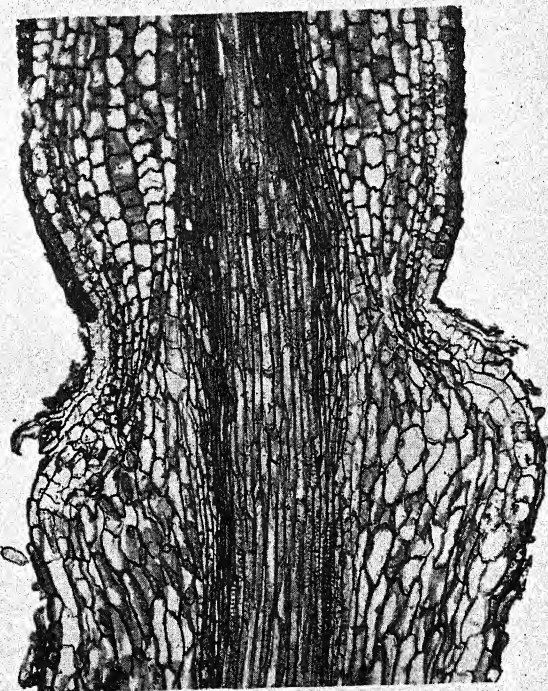
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the cells of the terminal meristem divide, adding new cells to the root cap distally and to the epidermis, cortex, and stele proximally (Hayward and Long, in press). The root cap is acute to sub-acute, and the outermost cells have thick heavily suberized or lignified walls. As the root elongates the peripheral cells of the root cap are lost, and the underlying ones form the suberized protective layer (fig. 2).

Under the influence of environmental factors which induce dormancy, the cells of the epidermis adjacent to the root cap become filled with deeply staining substances, and a similar condition develops in the cells of the hypodermis. There is a progressive extension of this reaction that involves one or two layers of root cap cells in a zone lying two or three cell layers outside the meristematic region (fig. 3). The cells of the deeply-stained layers become thick-walled, and there is a deposition of suberin on their inner faces. As a result, the cells of the distal portion of the root cap outside the suberized layer die (fig. 4). Coincident with these changes in the cells of the protective tissues, the activity of the meristematic cells diminishes. Continued differentiation results in mature stelar elements adjacent to the much reduced meristematic region (fig. 5).

The root tips in the resting stage are brown with flattened root caps in contrast to the actively growing root tips that are white with conical caps (fig. 2, 5). Senescent root tips resemble those of resting roots and may become gelatinized as necrosis progresses.

The resumption of active growth by a dormant root is initiated in the meristematic cells of the growing point and the cells of the pericycle adjacent to it (fig. 7). The combined activity of these cells produces a broadened growing point which forms new root cap cells inside the metacutis. As growth proceeds, the old root cap and metacutis are ruptured and displaced laterally, forming a shoulder of dead and broken cells which marks the point of renewed activity (fig. 8). There is no break in the continuity of the stelar elements, and the new ones are differentiated against the older ones, but at the point of resumption of activity the newly formed epidermis can be distinguished from the older epidermal cells by the degree of suberization and the presence of root hairs (fig. 9). The activation of the pericycle cells forces the endodermal layer toward the periphery of the root, and a separating zone is formed between the cortical parenchyma of the old and new growth. Cossmann (1939) describes a somewhat similar development as the formation of "a direct link of suberized cells . . . formed between endodermis and exodermis."

ANATOMICAL RESPONSES TO TREATMENTS.—Although brown roots, indicative of the dormant phase that commonly occurs in the ontogeny of the citrus

seedling, may resume active growth and form new white tips under normal circumstances, they will not do so if sufficiently high salt concentration and high pH values are maintained. In such cases, the inhibiting effects of high concentrations of chloride salts and low hydrogen ion concentration were reflected in an increased number of brown roots and a reduction in white root tips (table 2). In some instances, no white root tips were found on seedlings grown at the highest chloride level. In the most severe treatments, the brown inhibited condition was frequently followed by a breakdown of the meristematic tissue of the root and death of the seedling.

The histological picture accompanying progressive inhibition involves: (1) the reduction of meristematic activity in both the growing point and the pericycle zone, (2) the continued maturation of the stelar tissues until they abut the meristematic zone, and (3) a marked increase in the deposition of suberin on the walls of the epidermal, hypodermal, and root cap cells.

The inhibition of meristematic activity results in the formation of fewer cells by the histogens and a slower growth rate in the cells produced. In the pericycle, the potential cortical cells may not enlarge to the size they attain in the base nutrient solution, and in consequence the root may be smaller in diameter than those of the control plants. The stelar tissues continue to differentiate, and mature xylem and phloem elements ultimately abut the reduced meristematic zone thus eliminating the regions of elongation and differentiation that usually occur between the meristem and the mature primary tissues of the stele. This means that the processes of cell differentiation and maturation have proceeded at a relatively more rapid rate than that of cell division.

Another effect of high chloride and low hydrogen ion concentration is the reduction of pericycle activity in the formation of lateral roots. Except in the B series (unaerated, high pH value), there was a highly significant reduction in the number of lateral roots formed at the high chloride levels (fig. 1) (table 2).

Suberization of the walls of epidermal and hypodermal cells which normally accompanies dormancy was accentuated under high chloride conditions, and this was more pronounced in cultures where high chloride concentrations and high pH values were combined. The retardation in meristematic activity and consequent reduction in the production of new root cap cells, plus the development of a heavily suberized metacutis, results in a blunt root cap with few peripheral cells. One or two layers of cells may remain between the metacutis and the dermatogencalyptrogen layer (fig. 5).

THE DEVELOPMENT OF ROOT HAIRS.—The effect of hydrogen ion concentration, temperature and aera-

Fig. 6-9.—Fig. 6. Longitudinal section of semi-dormant root showing reduced growth activity.—Fig. 7. Root tip showing resumption of activity of the meristematic and pericycle cells.—Fig. 8. Longitudinal section of portion of root showing collar formation at point where growth activity was resumed.—Fig. 9. Longitudinal section of root at point of growth resumption showing the blocking off of the older cortical cells from the new. The old epidermis is heavily cutinized and root hair primordia have been formed by the younger epidermal cells.

TABLE 3. *Frequency of root hairs.*

Series	Salt treatments	None	Few	Many
A (pH 5.5-6.0, unaerated)	1—B. N.	17 ^a	50	33
	2—50 m.e. Cl ⁻ /l.	0	58	42
	3—100 m.e. Cl ⁻ /l.	31	50	19
AO (pH 5.5-6.0, aerated)	1—B. N.	6	31	63
	2—50 m.e. Cl ⁻ /l.	40	20	40
	3—100 m.e. Cl ⁻ /l.	92	8	0
B (pH 7.5-8.0, unaerated)	1—B. N.	81	19	0
	2—50 m.e. Cl ⁻ /l.	89	11	0
	3—100 m.e. Cl ⁻ /l.	77	23	0
BO (pH 7.5-8.0, aerated)	1—B. N.	63	37	0
	2—50 m.e. Cl ⁻ /l.	100	0	0
	3—100 m.e. Cl ⁻ /l.	100	0	0

^a Percentage of total number of root tips examined.

tion on root hair formation has been noted by Girton (1927). Under the conditions of his experiments, he found the most favorable conditions at pH 5.0 and 33°C. and demonstrated that continuous aeration greatly enhanced production. Cossmann (1939) observed that the length and development of root hairs was "directly affected by soil moisture conditions."

With respect to hydrogen ion concentration, our results confirm those of Girton. Seedlings growing in solutions maintained at the lower pH values (5.5-6.0) produced more root hairs than those growing in solutions having equivalent amounts of chloride salts but with pH values ranging from 7.5 to 8.0. Although low hydrogen ion concentration was the most important factor in inhibiting root hair formation, high concentration of the chloride ion was also a vital one. With one exception (the 100 m.e. chloride culture in the B series), there was a significant reduction in the number of root hairs at the high chloride levels. No root hairs occurred on plants in the aerated cultures at the high pH level in the 50 and 100 m.e. chloride solutions. In the base nutrient cultures, more root hairs were produced by the aerated than the unaerated plants as noted by Girton, but at both levels of hydrogen ion concentration, the high chloride

seedlings in the aerated cultures produced fewer root hairs than those in the unaerated solutions (table 3).

The treatment also influenced the length of root hairs. In general, the root hairs formed by this variety of orange are tubular, although some papillate, spatulate, and irregular forms do occur. In no instance were long root hairs developed, and under the most favorable conditions they seldom exceeded 100 μ in length. High hydrogen ion concentration favored development of the longest root hairs, but they were observed only in the control cultures of the A and AO series. The depressant effect of chloride was indicated by the shorter root hairs found in the 50 m.e. chloride cultures in both the A and AO series, and at the 100 m.e. level of the A cultures. At the high pH values, the development of root hairs was limited to the formation of primordia confirming Girton's results with respect to the effect of low hydrogen ion concentration.

The persistence of root hairs of the Valencia orange has been noted in a previous report (Hayward and Long, in press). The root hairs may become suberized and lignified, and in such cases persist for some time, although it seems unlikely that they remain functional. We have noted cases where

TABLE 4. *Mortality data.*

Series	Culture and treatment	Mortality no. of plants	Per cent of culture	Ave. no. days full treatment to death
A (pH 5.5-6.0, unaerated)	1—B. N.	0	0	0
	2—50 m.e. Cl ⁻ /l.	1	4	14
	3—1-00 m.e. Cl ⁻ /l.	6	25	36
AO (pH 5.5-6.0, aerated)	1—B. N.	0	0	0
	2—50 m.e. Cl ⁻ /l.	0	0	0
	3—1-00 m.e. Cl ⁻ /l.	13	54	47
B (pH 7.5-8.0, unaerated)	1—B. N.	0	0	0
	2—50 m.e. Cl ⁻ /l.	3	12.5	42
	3—1-00 m.e. Cl ⁻ /l.	13	54	44
BO (pH 7.5-8.0, aerated)	1—B. N.	0	0	0
	2—50 m.e. Cl ⁻ /l.	2	8.5	49
	3—1-00 m.e. Cl ⁻ /l.	13	54	48

root hairs have remained intact until the underlying hypodermal layer has divided periclinally to initiate a phellogen. The development of such hairs was more common with low hydrogen ion concentration than with high as might be expected on the basis of the relative degree of suberization of epidermal and hypodermal cells occurring under such conditions.

MORTALITY.—During the course of the experiment, 51 plants, or 17.7 per cent of the total population, died. Except for three seedlings in the A series that died at the end of two weeks while apparently in good condition, death was preceded by a slow progressive chlorosis and burning of the leaves accompanied by a gradual browning of the lateral roots. The color of the root tips changed from a glistening white to dull brown, and frequently this was accompanied by a gelatinization of the tissues of the root tip so that they appeared semi-translucent.

The major portion of the fatalities occurred between 35 and 50 days after the seedlings had received full treatment. No deaths occurred in the base nutrient cultures in either series and few at the 50 m.e. chloride level. At the 100 m.e. level, over 50 per cent of the population died in each of the AO, B, and BO treatments. The effect of high chloride was intensified by low hydrogen ion concentration. With high hydrogen ion concentration, the mortality rate at the high chloride level was significantly lower in the unaerated cultures (table 4).

SUMMARY

Valencia orange seedlings were grown in water cultures under three salt treatments: (1) a four-salt base nutrient solution containing no chloride except for a small amount occurring in the tap water (0.35 to 0.55 m.e./l.), (2) the nutrient solution plus 50

m.e. chloride per liter, and (3) the nutrient solution plus 100 m.e. chloride per liter. Chloride was supplied as NaCl 50 per cent, $MgCl_2$ 25 per cent, and $CaCl_2$ 25 per cent. Solutions were adjusted to pH 5.5–6.0 in one series and to pH 7.5–8.0 in the other. Half of the cultures of each series were aerated.

Citrus roots may exhibit alternating phases of activity and dormancy in their usual growth cycle. When in the dormant condition, meristematic activity is diminished and the walls of the cells of the epidermis and root cap adjacent to the meristem become suberized, forming a metacutis.

The anatomical response of the root to high chloride concentrations and high pH values resembles that of dormancy, but may be accentuated. In extreme cases, the root may remain in the dormant condition until death ensues.

Few root hairs developed on roots grown under conditions of low hydrogen ion concentration, and these were usually short or primordial in character.

The frequency and length of root hairs was reduced by high concentrations of chloride salts.

Chlorosis and tip burn of leaves may be induced by high chloride or low hydrogen ion concentration; the most severe symptoms occurred when the two treatments were combined.

Growth was inhibited under high chloride treatment and high pH values, fewer leaves and lateral roots being produced. The plants were shorter and lighter under such conditions.

Mortality was highest at the 100 m.e. chloride level in all series; few plants died under the 50 m.e. chloride treatment and none in the control cultures.

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PASSAGE OF AIR THROUGH PLANTS AND ITS RELATION TO MEASUREMENT OF RESPIRATION AND ASSIMILATION¹

Violette F. C-Glasstone

DURING THE course of preliminary respiration experiments with entire plants, it was found that air passes readily through the tissues either upward or downward. Since this finding was of some importance in connection with work on respiration, the results obtained were thought to be of sufficient general interest to justify a report. Plants in earthenware porous pots filled with soil, the surface of which was covered with a low-melting-point hydrocarbon wax mixture, were used. Particular care was taken to see that the soil was completely covered and that a good seal was made around the stem. While the wax was still fluid, a glass respiration chamber was placed over the shoot, which was thus completely enclosed; the system was apparently airtight as soon as the wax set hard. Air from a compressed air main was first freed from carbon dioxide and then passed into the respiration chamber; it left via an exit tube which led to a carbon dioxide absorption tower and thence to a vacuum tap. The only other opening to the respiration chamber was for a manometer, to indicate the pressure; by manipulating the compressed air and the vacuum valves, a steady rate of flow at atmospheric pressure was maintained. In testing the apparatus for leaks, a porous pot containing soil but no plant was poured with wax as described and the chamber sealed into place. The apparatus proved to be perfectly airtight; and, in fact, the manometers were so very sensitive that special precautions had to be taken to minimize the inevitable changes in vacuum or air pressure.

When experiments were made with pots containing plants, negligible and zero amounts of carbon dioxide were taken up in the absorption towers; this result suggested that no respiration was taking place, a conclusion which was obviously incorrect. It was noticed, however, that the manometers attached to the chambers containing the plants had ceased to show the sensitive movements, since use of either vacuum or compression line produced only slight positive or negative pressures. Similar results were obtained by using plants from which the shoots had been excised, leaving only cut stumps protruding through the wax. These facts suggested that air was passing right through the plants.

Confirmation of the fact that air was able to pass readily through plants was obtained by replacing the porous pot with a non-porous glass container. After a few minutes, equilibrium was attained and the manometers became exceedingly sensitive. Under these

conditions, normal respiration measurements could be made.

EXPERIMENTAL METHOD.—Experiments on the rate of passage of air through plants were carried out in the following manner. The specimen was placed in a porous pot with a respiration chamber sealed over the shoot as usual. A manometer containing *n*-butyl phthalate was fixed in position, and the only other opening was occupied by an exit tube leading first to a flow meter, next to a 20-liter glass jar which acted as a storage tank, and then to a T-tube connecting either to the vacuum tap or compressed air tap as desired. The vacuum tap was turned on until the manometer liquid just began to move; after a time the levels were noted and the suction increased. This procedure was continued until the maximum rate of flow measurable on the flow meter was reached. Analogous experiments were carried out using compressed air, instead of vacuum, and thereby reversing the direction of air flow. The levels in the manometer and flow meter at any rate of flow could be held stationary and the flow of air continued apparently indefinitely.

RESULTS.—Table 1 shows a typical set of readings obtained with a plant of *Nicotiana sylvestris* Speg. and Comes, of which the fresh shoot weighed 28.5 gm. and the area of under surface of leaves was about 878 sq. cm.

It will be observed from the figures in the last column that there was an approximate proportionality between the rates of air flow and the difference of pressure between the experimental chamber and the external atmosphere as measured by the manometer. The proportionality constant expressed in liters of air passing through the plant per hour per cm. of *n*-butyl phthalate is called the "rate coefficient" throughout this paper.

Similar results were obtained with all the plants tested: the data are summarized in table 2. The observations for each plant were reproducible over many hours; and where, as will be noted later, certain changes took place in the rate coefficient, these effects were also reproducible.

In some plants the rate coefficients were the same for either direction of flow, while in others there were differences in the two directions, *i.e.*, from leaf to root and root to leaf, respectively. Wherever two rate coefficients are given in column 4, as for example Oriental Poppy 5.5–35.0 liters per hour per cm. *n*-butyl phthalate, the air passed through the plant at the highest value for low rates of flow. When attempts were made to exceed a certain rate of flow, varying with each species, a resistance developed which diminished the rate coefficient until a certain pressure was attained. After a time at this high pressure, a new steady state was established, and the air

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TABLE 1. *Passage of air through Nicotiana sylvestris.*

	Rate of air flow (in liters per hour)	Difference in manometer levels (in cm. of butyl phthalate) ^a	Ratio of rate of air flow per cm. difference of pressure
Compressed air	7.5	0.5	15.0
	15.0	0.9	16.6
	22.5	1.5	15.0
	33.0	2.2	15.0
	46.5	2.9	16.0
	58.5	3.7	15.8
Vacuum	7.5	0.4	18.7
	15.0	1.0	15.0
	22.5	1.5	15.0
	33.0	2.2	15.0
	46.5	3.3	14.1
	58.5	4.4	13.3

^a One cm. of butyl phthalate is equivalent to about 10^{-3} atm. pressure.

usually passed through the plant at the higher speed. In most of the plants which showed this effect, the change, at the high pressure, from slow to high speed was very sudden. The pressure at which the rapid change occurred was fairly constant for each species and could be repeated apparently indefinitely by allowing a period of time to elapse or by reversing the air current. For the purpose of determining

whether the presence or absence of water within the plant or soil was a possible factor, a zinnia plant was well watered before being used. The experiment was continued on the same plant for two days. No water was added during the period but, since it was left sealed in the chamber all the time, the gradual loss of water was not serious, and the plant remained in good condition to the end of the test. Quite early on

TABLE 2. *Rate of air flow through plants.*

Species of plant	Total shoot weight in gm.	Total leaf area in cm. ²	Rate coefficient expressed in liters per hour per cm. n-butyl phthalate		
			Actual	Per gram of fresh shoot weight	Per square cm. of leaf area
<i>Nicotiana tabacum</i> var. Samsun...	52.5	1,150	150	2.9	0.130
	27.0	650	50	1.9	0.077
<i>N. sylvestris</i>	28.5	878	15.3	0.5	0.017
<i>N. glutinosa</i>	28	570	5.5	0.2	0.0096
Zinnia, Golden Gem:					
Vacuum	18.5	465	6.5-30 ^a	0.35-1.6	0.014 -0.065
Compressed air	18.5	465	9 -30 ^a	0.5 -1.6	0.019 -0.065
Poppy, Oriental	5.2	160	5.5-35 ^a	1.0 -6.7	0.034 -0.22
Aster, Shell Pink.....	14.5	639	7.5-13 ^a	0.5 -0.9	0.012 -0.02
Dianthus, China Pink.....	13.0	265	2 -20 ^a	0.15-1.5	0.0075-0.075
Crimson clover:					
Vacuum	24.0	760	85	3.5	0.11
Compressed air	24.0	760	55	2.3	0.07
Cabbage, Early Jersey Wakefield:					
Vacuum	52	1,002	6.5	0.125	0.0065
Compressed air	52	1,002	5-15 ^a	0.96-0.29	0.005-0.015
<i>N. rustica</i> :					
Vacuum	50.1	809	3.75	0.075	0.005
Compressed air	50.1	809	8	0.16	0.1
Cucumber, Henderson:					
Vacuum	18	447	10	0.55	0.022
Compressed air	18	447	1.5	0.08	0.0034
Tomato, Bonny Best:					
Vacuum	16	300	5	0.31	0.0167
Compressed air	16	300	1.5	0.09	0.005
Bean, Golden Cluster.....	13.6	394	4-12 ^a	0.3-0.9	0.01 -0.03
Wheat, Marquis Spring.....	6.5	253	4- 6.5 ^a	0.6-1.0	0.016-0.026

^a The explanation for the two rates is given in the text.

the first day the rate coefficient for passage of air from root to leaf suddenly increased to the high value of 30 l./hr./cm. pressure; while the low value of 9 l./hr./cm. pressure continued for the passage of air in the opposite direction. It was demonstrated repeatedly that reversal of air flow, or the passage of time, allowed a resumption of the resistance to the flow of air from root to leaf, and that the sudden change in rate coefficient always occurred at the same pressures. It was not until the latter half of the second day, in the case of the flow in the direction from leaf to root, that the rate coefficient began to increase gradually until it approached that of the flow in the opposite direction, i.e., 30 l./hr./cm. pressure.

In the case of *Nicotiana sylvestris* the leaves grow in a rosette, and the wax was poured so as to flow into the heart of the rosette and to cover all the leaf bases: no stem was above the surface of the wax. It was, therefore, certain that the air must have passed through the leaves. Other experiments were devised to expose either stem, root, or leaf only above the wax surface.

Plants of sunflower, tomato, *Datura* and tobacco were grown until the leaves from the lower part of the stem had died back naturally; the upper portion of the stem with leaves was cut off and the cut surface sealed with wax. These plants were used with the stump varying from 18–25 cm. long protruding above the wax. The rates of passage of air showed a proportionality as before, but the rate coefficients were considerably lower at 1 to 2 l./hr./cm. The stems were not able to allow a flow of air above an average of 10 l./hr. The tobacco stem, however, was exceptional, in that it would allow an air current of 60 l./hr. to pass from top to base with a rate coefficient of 8 l./hr./cm., if sufficient time was allowed for adjustment. The highest air flow in the reverse direction was 15 l./hr. with a coefficient of 3.5 to 4 l./hr./cm.

For roots only, tobacco plants which had been grown in nutrient solution were planted in sand, with some of the root above the surface; the stem and leaves were removed by cutting through the root region. Also the roots of dock plants, planted in soil, were exposed; the leaves and stem were cut away and the whole was waxed over, leaving the cut surface of the root uncovered. The rate coefficient for 3 cm. of exposed tobacco root was 30 l./hr./cm.; for the dock root with cut surface alone exposed it was 3 l./hr./cm.

For leaf only, the whole of a tobacco plant was waxed over except for one leaf of about 50 sq. cm. area. Even this small area allowed air to pass through in either direction at 3 l./hr./cm.

Although different plants exhibited certain differences of behavior, the experiments established the fact that the application of relatively small pressures will permit large volumes of air to pass through plant tissues of various kinds. The particular rate coefficient may, as with zinnia, be determined by the amount of water present in the plant or soil. The size

of the plant is probably a determining factor (cf. the two tobacco plants), and so also is its age (cf. the seedlings of bean and wheat). It appears justifiable to conclude, however, that individual and specific differences in plants are largely differences of degree.

In order to test the airtightness of the wax seal around the stem and respiration chamber, tobacco plants were prepared as usual. Before the readings were observed, however, water was poured on to the top of the wax seal, making certain that the level of the water was above the level of the wax both on the stem and around the edge of the chamber. No bubbles of air came through the water, although the tests were repeated several times on different days and with different specimens. Several depths of water were tried on each occasion, and at all rates of flow from the lowest to the highest the readings were comparable to those already given, indicating clearly that no leak occurred in the wax seal, and that the air was passing through the plant.

DISCUSSION.—In these experiments no attempt was made to determine the actual path taken by the air through the plant. The experimental results are closely related to the facts demonstrated by Zimmerman *et al.* (1931) on the very rapid passage of gases, particularly ethylene, into and out of plants; to the ventilation of tree trunks, investigated by McDougal (1936); and also to the observation of Cannon (1925) in relation to roots and the aeration of soil.

The work described in this paper proves the ability of air to pass in either direction through the entire plant, and presumably through isolated or attached organs such as leaves. In the case of some plants, especially tobacco, very high rates of air passage were demonstrated with almost undetectable changes of pressure. This result was important in connection with the measurement of quantities of gases liberated or assimilated by plants.

SUMMARY

Seventeen species of plants were examined and found to permit the passage of air through their tissues both in the direction of leaf to root and root to leaf. There was an approximate proportionality between the amount of air passing and the applied pressure; and the results were reproducible. It was noted that age, size, and moisture condition of the plant were factors which appeared to affect the rate of passage. It is suggested that, while individual and specific differences occurred in ability to allow air to pass through tissues, those differences are believed to be matters of degree. The rapid passage of air through plants was demonstrated in connection with the measurement of carbon dioxide produced in respiration.

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SEXUAL HORMONES IN *ACHLYA*. III. HORMONE A AND THE INITIAL MALE REACTION¹

John R. Raper

THE ACTIVITY of hormones in initiating and coordinating the sexual reproductive process in *Achlya bisexualis* Coker and *Achlya ambisexualis* Raper has been demonstrated by the writer (1939a, 1939b, 1940a). The sexual process takes place in a number of stages (Raper, 1939b), and these different stages are definitely correlated with the production and activity of the several hormones. The successive production of the different hormones and the activities which they bring about constitute a chained reaction with each stage dependent on the preceding ones. The initiation of the entire sexual reproductive process is brought about through the production, by the vegetative mycelium of the ♀, of hormone A which induces the formation of antheridial hyphae on the ♂ mycelium.

This work has suggested a number of interesting and important problems for further investigations. Of these problems, the one which concerns the ultimate identification of the active substances, is perhaps the most significant. The logical starting point for such an investigation is to determine something of the properties of hormone A, since it initiates and controls the initial ♂ reaction upon which all subsequent stages are dependent. Equally important is the incidental fact that, of the substances involved, it is the easiest to obtain in sufficient quantity for the determination of those properties.

Before the isolation of the active substance could be effectively attempted, it was necessary to work out some quantitative test for the relative concentration of the hormone in any test sample. It was also necessary to determine the effects of various environmental conditions on the response of the ♂ test plant to the active substance in order that tests could be performed under optimal conditions for all factors except the unknown variable.

The scope of the present paper is, therefore, limited to include only the production of hormone A by the ♀ plant and the physiological aspects of the production of antheridial hyphae on the ♂ mycelium in

response to this substance. From the physiological data given below the necessary standardized conditions for a quantitative test for hormone A are apparent.

MATERIALS AND METHODS.—It was shown in an earlier paper (1940a) that the ♂ plant of *A. ambisexualis* reacts more intensely when placed in the water in which a ♀ of *A. bisexualis* has grown than in the filtrate from a ♀ of *A. ambisexualis*. It has not been determined whether this is due to the greater production of hormone A by the ♀ of *A. bisexualis* or to slight differences in the chemical nature of the hormones secreted by the two ♀ plants, the ♂ being more reactive to one than to the other. That the former is the correct explanation for the different degree of effect is probable, and for the purpose of the present study the ♀ plant of *A. bisexualis* has been used exclusively as a source of hormone A and the ♂ of *A. ambisexualis* as the test plant for this substance.

Source of hormone A.—The most suitable method for obtaining a supply of hormone A has been found to be large water cultures of the ♀ fungus. For the maximum yield of the hormone it is necessary to adjust conditions so that vegetative growth is vigorous. This requires temperature of 20-25°C., the presence of trace quantities of a number of inorganic salts (Raper, 1939b), a suitable nutrient, and equally important, the absence of toxic substances. While it is not absolutely necessary to remove all impurities from the water, the yield of hormone can be increased considerably through the use of Pyrex distilled water in preparing the medium. As a nutrient, hemp seed (*Cannabis sativa*) have been found to give the most satisfactory results, the hormone content of hemp cultures being 200-1,000 per cent greater than in similar cultures in which corn, rice, lentils, starch-peptone, etc., have been used as nutrients. This fact in itself is probably significant, and may eventually aid in the identification of the substance.

Closely reproducible solutions may be obtained by using 2 gms. of hemp seed per liter of glass distilled water to which small quantities of KH_2PO_4 , MgSO_4 , CaCl_2 , FeCl_3 , and ZnSO_4 have been added. No appreciable difference has been observed in the hormone content of cultures to which split seed were added as nutrient, the mold thus having a solid substrate (approximately 1 mycelium for each 5 cc. of water), and those provided with an aqueous extract

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of ground autoclaved seed. In the latter case it is necessary to inoculate by introducing a large number of zoospores, since the introduction of an agar block containing a few hyphal tips leads to the production of only a single spherical mycelium.

Air is bubbled through the cultures for two reasons: (1) to keep the water in constant movement so that the numerous mycelia will remain separate and (2) to provide a constant supply of oxygen which is essential for maximum growth.

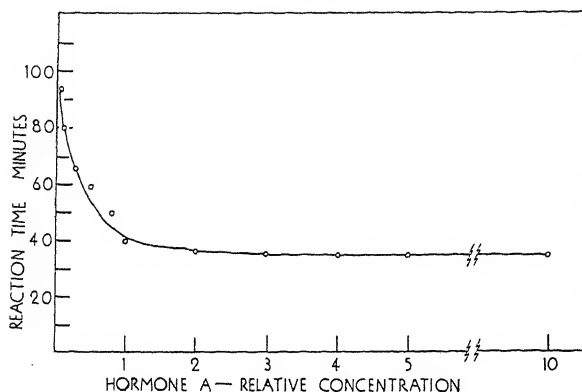


Fig. 1. Graph showing the relationship of concentration of hormone A to the elapsed time of production of antheridial hyphae after immersion of δ plants of *Achlya ambisexualis* in test solutions. On the abscissa unity is the untreated filtrate from the φ of *A. bisexualis* as described in the text.

At room temperature vegetative maturity of the mycelia is reached in such cultures on the fourth or fifth day after inoculation. The concentration of hormone A increases until about the fifteenth day of incubation.

Preparation of δ test plants.—Care must be taken to secure fairly uniform δ test plants. The simplest and easiest method that has been found is as follows. Ten very small agar blocks ($1 \times 1 \times 0.5$ mm.) containing hyphal tips of the δ plant are placed widely distributed over the bottom of a Petri dish, and on each block is placed one-half of a split hemp seed. The smallest amount of water that will barely cover the bottom of the dish is added, and the culture is then incubated under the desired environmental conditions for twenty-four hours. At the end of this time the mycelia are vigorously washed with a stream of water from a wash bottle and approximately 10 cc. of water is added to each dish. After forty-eight hours additional incubation, they are approaching vegetative maturity and exhibit maximum sensitivity to the active substance.

EXPERIMENTAL RESULTS.—Two possible criteria for measuring the intensity of the δ reaction to hormone A were recognized during previous work on the hormonal coordinating mechanism in *Achlya*. The first objectives, then, were: (1) to ascertain the relationship between the quantity of hormone A present and the intensity of the δ reaction which it induced

and (2) to explore the two possible means of determining the reaction intensity.

Time of reaction.—During the investigation of the sexuality of *A. ambisexualis* (Raper, 1940b), it was frequently observed in both water and agar matings that the time which elapsed between approximation of two compatible mycelia and the initiation of antheridial hyphal production was correlated with the quantity of mature sexual organs ultimately produced. Invariably where the initiation of antheridial hyphal development was delayed more than a few hours the final production of oogonia and oospores was scant. Since subsequent stages in the sexual reaction are dependent on and doubtless limited by the intensity of the reaction, it seemed probable that the reaction time of the δ plant would vary inversely with the concentration of hormone A.

To test this possibility the liquid in a 3-liter φ culture was concentrated in a reduced pressure still to one-tenth its original volume, and from this concentrate a series of solutions was made up by dilution to contain concentrations varying from one-thousandth to ten times that of the original filtrate. Into 10 cc. of each of these solutions were placed two 72-hour δ test plants. These test plants were observed at very frequent intervals with a dissecting microscope and the time of the first appearance of antheridial hyphae for each test solution was recorded. The results of one such experiment are given in figure 1. The hypothesis advanced above was borne out in a number of such series, for in all cases the δ plants in higher concentrations of φ filtrate (containing hormone A) reacted sooner than those in lower concentrations. However, there were two objections to this method of determining hormone A concentration. (1) In all save the very lower limits of the series the reaction time is changed so little by relatively great changes in concentration of the hormone that the test is not sufficiently critical. (2) It is exceedingly difficult to determine the exact moment when the small knobs, which will develop into the thread-like antheridial hyphae, first appear on the vegetative hyphae. This difficulty is increased when an extended series of solutions is tested and the number of observations increased.

Number of antheridial hyphae.—The second possible criterion of reaction intensity was the relative number of antheridial hyphae produced by a δ test plant. In a mating of two compatible plants the relative abundance of antheridial hyphae seems to be directly proportionate to the number of oogonia and oospores finally produced. Since hormone B is secreted by the δ plant only after the production of antheridial hyphae, and in all probability produced by the antheridial hyphae themselves, subsequent reactions are probably limited by the number of antheridial hyphae produced, the number of these organs in turn being limited by the quantity of hormone A.

To determine whether this was the case, a concentration series, similar to those used in the preceding experiments and prepared from the same φ concen-

trate, was made up and into each of the solutions were placed two ♂ test plants. At the end of two hours numerous antheridial hyphae had been produced on the test plants. The average number of antheridial hyphae on 3 mm. hyphal tips of the test plants was determined for each of the several concentrations. Three millimeters was the distance selected because of differences of distribution of antheridial branches on the ♂ hyphae in reactions of high and low intensity. When the number of antheridial hyphae is small, they are almost invariably located within 0.5 mm. of the hyphal tips, whereas, when a larger number are formed, they are distributed approximately equally along the entire length of the parent hypha. Actually a count of the number along the entire hypha might give more accurate results, but such determinations are complicated by the dense and tangled growth near the substrate.

For each determination antheridial branches were counted on ten hyphal tips chosen at random on each of the two test plants. Thus an average of twenty counts was used for each test.

The relationship between the concentration of hormone A and the production of antheridial hyphae on the ♂ plant is shown in figure 2. In this figure the reaction intensity, as measured by the number of antheridial hyphae/3 mm. hyphal tip, is plotted against relative concentration of ♀ filtrate where unity is the untreated filtrate from ♀ plants as described above.

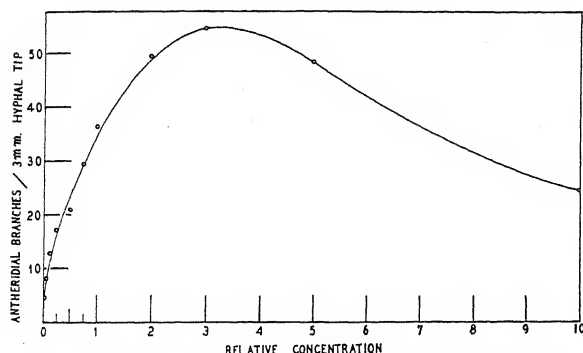


FIG. 2. Effect of hormone A concentration on the number of antheridial hyphae produced on the ♂ plant. Concentrations shown on the abscissa are identical with those in figure 1.

In lower concentrations small increases of the hormone result in considerable increases in the number of antheridial hyphae until an optimal concentration is reached at three times that of the original ♀ filtrate. That the reaction is less beyond this point of optimal concentration is doubtless due in part to an increased amount of solutes in the solutions (see below) as well as to a supermaximal quantity of the hormone. The quantity of ♀ filtrate used for each test (10 cc.) is only an arbitrary and convenient amount. The number of antheridial hyphae produced by a plant in a 1 cc. sample and that produced in 10 cc. is the same. The amount of hormone per unit

volume of water (concentration) determines the number of antheridial hyphae rather than the total amount of hormone available to the ♂ plant.

This method is preferable to that using the time interval between application of the hormone and the first appearance of visible effect for the following reasons: (1) To determine the quantity of antheridial hyphae produced it is not necessary to keep the test plants under constant observation; hence it is

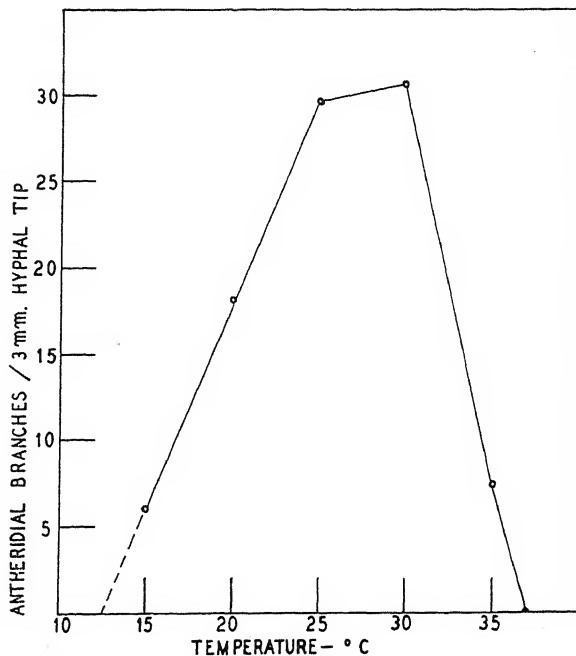


Fig. 3. Effect of temperature on the initial ♂ reaction (production of antheridial hyphae). The test solution for these determinations was untreated ♀ filtrate, represented by unity on abscissae of figures 1 and 2.

possible to work with a considerably larger number of tests. (2) Greater accuracy is possible, since, in measuring reacting time, it is difficult to determine the exact moment when antheridial hyphal formation is initiated. (3) Small changes in concentration of the hormone within the critical range bring about greater percentage differences in the number of antheridial hyphae produced than in the length of time required for their initiation.

The average number of antheridial hyphae on a hyphal tip of the ♂ plant forms the basis of the quantitative determination (biological assay) of hormone A after standardization of the various environmental conditions affecting it.

Conditions affecting the reaction of the ♂ plant to hormone A.—1. *Temperature.*—The range of temperature within which ♂ and ♀ plants of *A. bisexualis* and *A. ambisexualis* give intense sexual reactions is from 23–28°C. Vegetative growth of both these species is normal over a much wider range, from 15–40°C. The rate of growth, however, becomes progressively faster the higher the tempera-

ture. It seemed, therefore, expedient to determine the relationship between reaction intensity and temperature in order to ascertain the optimal temperature for the δ reaction as well as to clarify the discrepancy between temperature requirements for sexual activity and those for normal vegetative vigor.

A series of identical test solutions, each containing two δ plants, were placed in incubators with temperatures ranging from 15 to 37°C. At the end of two hours counts, as described above, were made of the antheridial hyphae on the δ plants from the different temperatures.

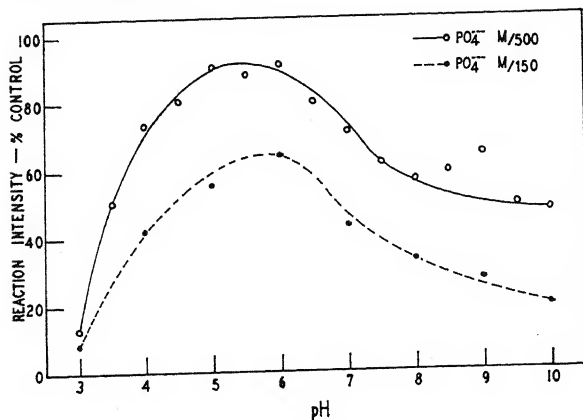


Fig. 4. Effect of hydrogen-ion concentration on the initial δ reaction. The unbuffered control (untreated φ filtrate) had a pH value of 6.2 and gave a reaction of 36 antheridial hyphae/3 mm. of hyphal tip. The maximal reactions in both buffered solutions are lower than the unbuffered control, and the more strongly buffered series (M/150 total phosphate) consistently gave less reaction than the weaker buffered series (M/500 total phosphate). Any dissolved electrolyte depresses the reaction in proportion to its concentration.

The relationship between temperature and reaction intensity is shown in figure 3. At 15°C. the reaction is slight, at 25 to 30°C. an optimum is reached, and for further increases in temperature there is progressively less activity. At 37°C. the δ gives no reaction whatever.

This relationship between temperature and reaction of the δ plant to hormone A brings out a number of interesting points. The limiting effect of high temperatures in many biological reactions is caused by the permanent injury of the reacting tissue or material. Here this cannot be the case, for the maximal temperature for vegetative vigor is considerably higher than the maximal for the production of antheridial hyphae. On the other hand the limiting effect at 37°C. cannot be the destruction of hormone A, for it will withstand a temperature of 100°C. for several hours without detectable loss of activity. The question naturally arose whether the ability of the δ plant to react to hormone A was merely inhibited for the duration of supermaximal temperature or whether this ability was thereafter permanently lost. If this ability were not completely destroyed, would

the δ plants be capable of reaction immediately when returned to optimal temperature, or would re-action ability return gradually only after a lapse of time?

In order to elucidate the phenomenon the following simple experiments were performed. Several δ plants were placed in an incubator at 37°C. for five hours and then returned to 25°C. and tested immediately by immersion in 10 cc. samples of φ filtrate. Plants which had remained at 25°C. were tested simultaneously. The average number of antheridial hyphae of the plants of the two series was approximately the same, well within the limits of counting error. As a further control, two of the plants placed in the 37°C. chamber were immersed in the test solution of hormone A during heating and during this time no reaction resulted. These plants were then returned to 25°C. in the test solution and counts of the antheridial hyphae made at the end of two hours. The intensity of the reaction in these plants was approximately equal to that of the other tests and to that of the 25°C. controls.

These results show (1) that the sensitivity of the δ plant is only temporarily inhibited by supermaximal temperature, (2) that no permanent injury to the mechanism of reaction results from such heating, and (3) that there is immediate recovery of reaction ability when plants are returned to less severe conditions. It is also apparent that a close control on temperature must be maintained in order that the test might be used as a quantitative test (biological assay) for hormone A.

2. *Hydrogen-ion concentration and the effect of dissolved salts.*—Hydrogen-ion concentration within wide limits has previously been shown (Raper, 1939b) to have little effect on the entire sexual process of *A. ambisexualis*. However, for the purpose of quantitatively determining hormonal concentrations any appreciable hydrogen-ion effect should be accounted for and adjustment made to the optimal pH.

Two series were made up using untreated φ filtrate as a base. The first series contained phosphate buffers of 1/500 Molar concentration and ranging from pH 3 to 10. The second series, similar in range, contained phosphate buffers of 1/150 Molar concentration. The untreated φ filtrate, which had a pH value of 6.3, was used as a control for both series. Two 72-hour δ test plants were placed into each of the different solutions, and counts of the antheridial hyphae were made at the end of two hours.

The results of these two series of tests are given in figure 4, the relative intensity (as compared to the unbuffered control) of the reactions in the various solutions being plotted against the concentration of hydrogen-ions. The optimum hydrogen-ion concentration for the reaction lies near pH 6. This optimal pH is extremely close to that of the unbuffered φ filtrate, and of a large number of φ filtrates for which the hydrogen-ion concentrations were determined none varied from pH 6.3 by more than 0.2 units. Thus in many cases adjustment of hydrogen-ion concentration is not necessary.

In these tests another limiting effect is shown which is probably more important than the effect of hydrogen-ion concentration. A comparison of the curves in figure 4 shows that the reactions in the weaker buffered solutions (M/500) were consistently higher than the reactions for the same pH values in the stronger (M/150) solutions. Further, the maximum in each series is significantly less than that of the unbuffered control at approximately the same level of hydrogen-ion concentration. A point of complete inhibition of the δ reaction is reached before salt concentration becomes toxic to the test plants.

This depressing effect of dissolved salts complicates the determination of hormone A concentration. Many of the samples which need to be assayed for hormonal content in the course of working out a procedure for the isolation of the active substance contain salts in greater concentration than will allow of quantitative determination of the hormone. Such samples, then, must be freed of their salts before the tests are made. This can usually be accomplished by precipitating the salts by the addition of acetone in which the hormone is readily soluble, or by extracting the active component with ethyl ether. Either of these methods results in a sample sufficiently free of inhibiting dissolved inorganic compounds to be assayed directly.

3. *Variation in sensitivity of δ plants.*—One of the first experiments performed on the φ filtrate was to determine the stability of the hormone over a period of time. In tests made at four-hour intervals δ plants showed progressively less reaction during one day. The same φ filtrate tested the following day with plants from the original δ culture induced reactions at first equal to the maximum of the day before, the reactions of the δ plants then decreased at subsequent times of testing. These observations indicated a diurnal rhythm in sensitivity of the δ plants to the hormone. However, preliminary experiments showed that the time of maximum sensitivity varied from day to day. A similar condition has been known for years in the reaction of *Avena* coleoptiles to auxins (Kogl, 1933; Kogl, Haagen-Smit, and van Hulssen, 1936; Juel, 1936. Cited in Went and Thimann, pp. 49-51, 1937). A comparison of the variation curves of *Achlya* sensitivity has been made with those of *Avena* tests performed concurrently by Went *et al.* Although the curves of variations of the two plants are similar they do not "fit," for the maxima in the two cases occur at different times and the lengths of time for the complete cycles in the two tests are also different.

Since the reaction of the δ plant to the same φ filtrate varies so drastically from one time to another and the rhythm is not a rigid 24-hour one, the quantitative test for hormone A must also be standardized in respect to that variation. The ideal solution to the problem would be the discovery of means of eliminating or at least reducing the variation to a low percentage of the maximal reaction. With this

objective in view a number of environmental factors have been eliminated as the controlling agents.

Naturally the first factors to be suspected were light and temperature. Accordingly δ test plants were grown and tested in a room with continuous orange light, the temperature being constantly maintained at 25°C. The first day's tests under these conditions showed the inadequacies of these measures,

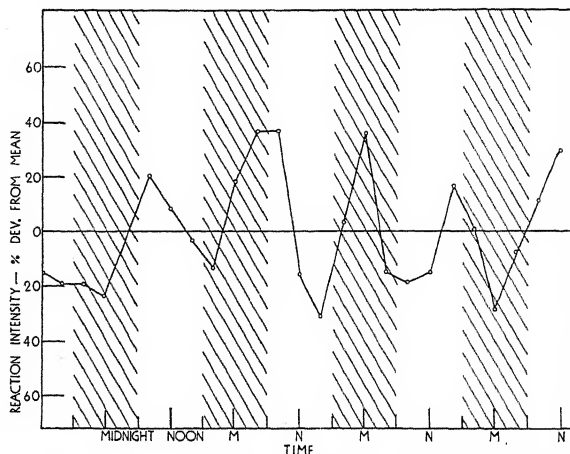


Fig. 5. Variations in the intensity of reaction of δ plants of *A. ambisexualis* to a standard solution of hormone A during 96 hours. Cultures of the δ test plants used in this series were made at noon three days before they were used in the tests, and the change to new plants was made each day at 12:00 midnight. It is apparent from the curve above that small differences in the age of the δ plants affect neither their activity nor the variations of their response to hormone A at different times.

for the variations were as great as at room temperature with alternating light and darkness. Stock δ cultures were then grown under these conditions for a month and subcultures were then tested in portions of φ filtrate at four-hour intervals for 96 hours. The results of these tests are shown in figure 5. During the 96-hour period five complete cycles were observed, an average of nineteen hours between comparable points on the different cycles. On the third day of the experiment the average number of antheridial hyphae was 12.4 at 4:00 P.M., and eight hours later at 12:00 midnight a reading of 24.1 was recorded, an increase of almost 100 per cent. Even greater variations have been repeatedly observed in a single sample of φ filtrate.

Next in the order of suspects was the effect of minor changes in the concentration of noxious gases in the atmosphere. The first experiments to determine whether or not changes in the air were responsible for the variation were performed in the following manner. Male test plants were grown in each of two constant temperature rooms (25°C.). In one of the rooms the air stream from the air conditioning system was passed through a charcoal filter while in the other room the air was unfiltered. Tests were then

made in the usual manner at intervals of three hours during a 24-hour period.

The plants in each of the two rooms varied in their reaction to the standard hormone solution, the variations reaching a maximum of 140 per cent. The shapes of the two curves fit closely, although the series of plants in the room with the filtered air was uniformly slightly higher than in the room with the untreated air.

Since there was an appreciable difference in the two series indicating that composition of the air affected the test it seemed desirable to extend the study. Plants were then grown in a closed chamber into which thoroughly washed air was led from a tank; thus during the experiment there would be no variation in the composition of the air around the cultures. The plants grown under these conditions were then tested in the constant air chamber at four-hour intervals concurrently with controls outside.

Again the reactions of the two series followed the usual pattern varying considerably during the period of testing, but with only minor differences between the reactions of plants of the two series at any one time.

By this time it was apparent that the rhythmic variation of sensitivity of the δ plants was not to be eliminated by simple control of environmental factors. During the time occupied by the preceding experiments the cycles of variation were found to be very irregular. At one time an average of seventeen hours elapsed between maximum reactions, at others as high as thirty hours elapsed. Since the variation could not be eliminated the next best solution to the problem was to stabilize its cycle at twenty-four hours so that quantitative tests could be made at the same time on different days. Two attempts to accomplish this end were made.

The first was to grow and test the δ plants in a constant temperature room (25°C.) with twelve hours of brilliant illumination alternating with twelve hours of total darkness. Plants grown and tested in constant temperature and continuous orange light were used as controls. For a 48-hour period of testing there was no difference in the reaction of the plants of the two series. During this experiment the variation of both controls and alternating dark-light plants followed a 24-hour cycle, but it must be considered as coincidental since the cycle of variation under constant illumination varies from seventeen to thirty hours.

A second attempt was then made to stabilize the cycle at twenty-four hours. The heating unit of a 25°C. room was equipped with a time switch, so that during twelve hours the temperature remained at 25°C. but dropped to approximately 20°C. for the alternating twelve hours. The lighting arrangement was left as in the preceding experiment with brilliant illumination during the period when the temperature remained at 25°C. and with the room in darkness when the heating unit was shut off. Male plants were then grown and tested under these conditions each day for five consecutive days. A small degree of success

was achieved by these measures, for the first test made after the temperature was brought up to 25°C. each day was the highest for that day with a rapid decrease in antheridial hyphae at subsequent times of testing. However, the difference between the maxima of the several days was still too large to use the number of antheridial hyphae directly as a measure of hormone concentration. Further work in which the temperature is more drastically changed and better controlled might possibly result in the complete stabilization of the cycle on a 24-hour basis. This has not yet been attempted.

It has been clearly shown above that, although environmental conditions may affect the sensitivity of δ plants, controlled conditions of the test will not eliminate the cyclic changes.² Apparently the variability is an inherent property of the plant and is affected only indirectly by external conditions. The quantitative test for hormone A is further complicated, since it has not been possible either to control or to stabilize the variation of the δ plant.

Two means of dealing with the situation have been used and both are inadequate. Tests to give quantitative results should be made while the δ plants react with maximum sensitivity. Such a procedure is obviously impractical, since the time of the variation cycles also varies.

"Calibration" of the δ plants by means of a standardized solution containing hormone A has been used. When the variation from maximum sensitivity is slight such a method is satisfactory, but its accuracy decreases with greater variations, for the relationship between the number of antheridial hyphae and the concentration of hormone A is not a linear function at any concentration level of the hormone. Thus it has been necessary to repeat those tests in which the reaction is less than 50 per cent of the maximal. When frequent tests are made the time of maximal sensitivity is approximately known for each day and the number of repeat tests is not excessive.

The biological assay and definition of the physiological unit of hormone A.—It has been shown above that the intensity of reaction of the δ plant to hormone A is influenced by a number of environmental conditions in addition to the amount of hormone A present. These factors then must be standardized if a test for activity is to be quantitative.

A physiological unit of hormone A is that amount of hormone/cc. required to produce an average of ten antheridial hyphae/3 mm. of vegetative hyphal tip of 72-hour test plants (1) at the time of maximal δ sensitivity, (2) at 25°C., (3) with the pH adjusted to a value of 6.0, and (4) in the absence of inhibiting salts.

The original untreated φ filtrate used in the tests to show the relationship between hormone concentration and reaction intensity contained, on the basis of

² After the completion of the work dealt with in this paper a means of controlling this variation has been found. This control and related matters will be described in a future account.

this definition, 18 units of hormone per cc. Thus the concentration of hormone which induces the maximal reaction in the δ plant is 50 to 60 u/cc. of test solution. However, at this level changes in concentration bring about very small differences in the number of antheridia. The curve is much more critical at lower concentrations. The standard solution used to "calibrate" the plants in quantitative tests contained 6 u/cc. the lowest value in the critical portion of the curve where counting error could be kept below 5 per cent.

EFFECTS OF KNOWN CHEMICAL COMPOUNDS.—Hormone A is secreted in very small quantity by the φ plants of *A. bisexualis* and in exceedingly low concentrations brings about the production of antheridial hyphae on δ plants. Because of the small quantity of the hormone produced by the φ plant, the difficulties of obtaining sufficient material for chemical work were recognized at an early stage of this work. During the past two years approximately a hundred plant and animal tissues and products have been tested in the hopes of finding another source of the material. The results in all cases have been negative. When the number of materials tested and the chemical richness of certain of them (i.e., urine, yeast extract, hay infusion, etc.) are considered, it is clear that the substance responsible for the reaction of the δ plant is no commonly occurring compound. The continuation of testing materials at random might eventually locate a source of the hormone, but enough have already been tried to make this procedure of doubtful value.

In addition to natural products a large number of known and chemically pure compounds have also been tested for activity. Two groups of compounds have been found in which certain members show some activity.

The saturated dicarboxylic acids comprise the first of these groups. Those containing from 2 to 10 carbon atoms have been tested. Three of these, malonic, glutaric, and pimelic acids, induced the formation of a limited number of antheridial hyphae. The number of antheridial hyphae produced in each case was small, but there was no doubt as to their identity as such. Of these three the reaction given by glutaric acid was the most intense, while the reactions given by malonic and pimelic acids were slightly less and approximately equal. It is remarkable that each active member has an odd number of carbon atoms in the chain and that the longer odd-numbered as well as all of the even-numbered members were inactive. A number of closely related acids have been tested, fumaric, maleic, malic, tartaric, and citric, and in all cases the results were negative. That none of the three active acids is identical with hormone A is certain, for in each case (1) the reaction is always slight, an average of 10–15 antheridial hyphae/3 mm. of hyphal tip being exceptional; (2) relatively high concentration (1 mg. to 10 cc. water) of the acids is necessary to get a reaction, and higher concentrations are toxic; and (3) the relationship of activity to pH is different from that of hormone A. For

pimelic acid the maximum lies at 4.5, for glutaric acid at 4.0 and for malonic acid at 3.5.

More significant than the effect of these three acids on the δ plant is their effect on the φ plant. When small quantities of either glutaric or malonic acid are added to the medium in which φ plants are grown, hormone A production is increased in proportion to the amount of acid added (fig. 6). A maximal effect

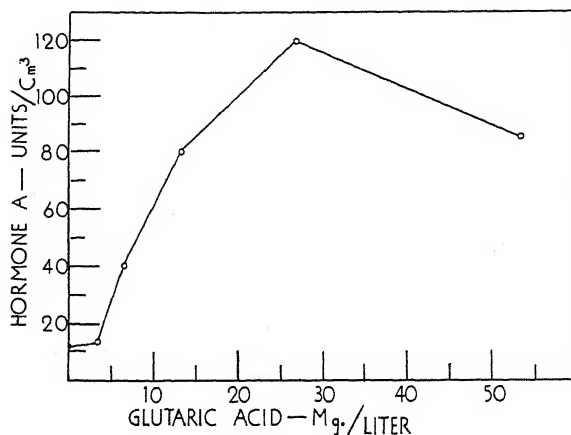


Fig. 6. Graph showing the effect of glutaric acid on the production of hormone A by the φ plant of *A. bisexualis*. These determinations of hormone concentration were made five days after the cultures were started. The concentration of hormone reaches a maximal value only after twelve to fourteen days' incubation of the cultures. The addition of malonic acid to the culture medium of the φ plant has a similar and slightly greater effect on hormone A production than glutaric acid.

is reached when 25 to 35 mg. are added to a liter of culture medium. A culture to which glutaric or malonic acid has been added in this concentration after growing a week contains frequently ten to fifteen times as much hormone A as a control culture lacking the acid. When the cultures are allowed to remain undisturbed for a longer period of time this increase continues until the cultures are about two weeks old, the hormone concentrations sometimes reaching almost a hundred times that of the control. The greatest concentration of hormone A yet attained has been in ten- to twelve-day-old 18-liter cultures with the φ plant growing on halved hemp seed and with 400 mg. of malonic acid added to each culture. In 80 cultures of this kind the average concentration of hormone was 1,330 hormone units in each cubic centimeter of filtrate. That it is an actual increase in hormonal concentration in the φ filtrate is shown by adding the acid to the control φ filtrate and immediately testing the mixture. Then the number of antheridial hyphae which are produced is less than the number that can be accounted for by the two factors separately. The acid is apparently used in the synthesis of the hormone. Whether it enters directly into this process or affects the process indirectly is not known.

Certain members of the second group, barbituric acid and related compounds, show much more activity when tested with ♂ plants than any of the dicarboxylic acids. Barbituric acid and hydantoin induce activity of low intensity. A number of substituted derivatives of barbituric acid have been tried in the test. Certain of these derivatives were completely inactive, while others induced much more intense reactions than unsubstituted barbituric acid. Actually one of the derivatives of the acid induced in the ♂ plant the most intense reaction yet seen, but a relatively high concentration (10^{-5}) of the compound is necessary before any reaction results.

Too few of the derivatives of barbituric acid have been tested as yet to correlate activity in inducing the formation of antheridial hyphae definitely with any particular structure or grouping of constituents within the molecule. The investigation of these compounds, however, is being continued and will be given in detail in a future account.

The barbiturates, unlike the active dicarboxylic acids, have no apparent effect on the ♀ plant and the production of hormone A.

SUMMARY

Techniques are described for culturing the ♀ plant of *Achlya bisexualis* as a source of hormone A and the preparation of ♂ plants of *A. ambisexualis* as test material for the ♀ hormone.

The relationship between hormone concentration and the activity of the ♂ plant has been determined. The number of antheridial hyphae produced in response to the hormone furnishes a satisfactory index of the reaction intensity, the number of antheridial hyphae being proportionate to the concentration of the hormone. A less critical test is the length of time required by the ♂ plant to initiate the production of antheridial hyphae. This method has been rejected because of the difficulties of determining the exact time of the initiation of the reaction.

The reaction of the ♂ plant is limited by a number of factors in addition to the concentration of hormone in the test solution. Optimal conditions of temperature and hydrogen-ion concentration have been determined. Light has no apparent effect on the reaction. Dissolved inorganic salts in the test solution have a depressing effect, and the reaction can be completely inhibited by increasing their concentration. The ♂ plant is shown to vary considerably in its sensitivity to hormone A, the variation being very irregular. Attempts to eliminate or minimize this variation as well as attempts to stabilize the variation cycles at twenty-four hours have been unsuccessful.

Standardized conditions are described for the quantitative determination (biological assay) of hormone A and the activity or physiological unit of the hormone is defined.

The effects of certain pure chemical compounds on the ♂ plant of *A. ambisexualis* and the rate of hormone A production by the ♀ of *A. bisexualis* are described and briefly discussed.

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EMBRYO CULTURE AN EFFECTIVE TECHNIQUE FOR SHORTENING THE BREEDING CYCLE OF DECIDUOUS TREES AND INCREASING GERMINATION OF HYBRID SEED ¹

W. E. Lammerts

ARTIFICIAL CULTURE of embryos in nutrient solutions has long been practiced. Hannig (1904) showed that after a certain stage of development unripe seeds of Crucifers may be removed and brought to maturity by artificial culture. Dieterich (1924) repeated and extended these experiments to include species in other families, and Laibach (1929) showed its advantages by successfully growing embryos of flax hybrids which ordinarily abort if left

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Grateful acknowledgment is made to H. B. Tukey who kindly supplied me with the information available in 1936 and to the Armstrong Nurseries for providing facilities and growing the seedling trees.

to ripen on the plant. Tukey (1933) applied the technique to the germination of early sweet cherry embryos which usually abort, and later (1934, 1938) reported successful culture of peach, apple, pear, and plum embryos. LaRue (1936) cultured immature embryos of *Lactuca*, *Taraxacum*, *Chrysanthemum* and *Zea*. Blake (1939) outlined a method of embryo culture different from that of Tukey and successfully cultured over 1,000 hybrid peach seedlings, many from varieties which normally have partially shriveled embryos difficult to germinate by ordinary stratification methods. Approximately 75 per cent of these seedlings fruited three years after the crosses were made.

In most of the above experiments, the artificial culture technique was mainly of value as a means of growing immature or anomalous embryos and defective seeds. During the past five years while developing a fruit-breeding program at Armstrong Nurseries, Ontario, California, several thousand peach, nectarine, and apricot seeds were artificially cultured. Germination and subsequent growth was compared with that obtained by the usual methods of stratification. The following data are presented to show that embryo culture may effectively be used in large scale commercial fruit breeding to shorten greatly the breeding cycle of deciduous trees and markedly increase the germination of hybrid seeds. Certain problems yet to be solved are discussed and suggestions made which may lead to their solution.

MATERIALS AND METHODS.—*Culturing of embryos.* In general the methods described by Tukey (1934) were used. However, the following changes in culturing technique were helpful in avoiding failures:

(1) The pits were removed from ripe fruit, sterilized in mercuric chloride 1–1,000 and stored at room temperature for three to four weeks before culturing the embryos.

(2) Hot water was used to make the calcium hypochlorite disinfecting solution.

(3) The embryos were then sterilized by placing them in this solution which was heated to 110° to 115° F. Thorough but gentle shaking and stirring of the solution is necessary to remove air bubbles from the surface of embryos. A surface tension reducer such as the 10 per cent Aerosol O. T. C. powder used at the rate of 1 gram per liter is helpful if the embryos are badly infected by fungi on the testas.

(4) A .9 per cent agar solution was used to avoid softening during hot weather. Bacto-Difco agar and Baker's chemicals were used.

(5) Vitamin B₁ at the rate of 1 mg. per liter proved helpful in stimulating root formation. Instead of glucose, .5 per cent sucrose for mature and 2 per cent for immature embryos was used.

(6) The culture bottles were placed in standard flats to a depth of about ½ inch in a layer of wet peat. They were then placed under greenhouse benches in subdued indirect light.

Methods used in growing of seedlings and young trees.—The embryos began to grow soon after placing them in the culture bottles and in two or three weeks were usually large enough to transplant. Meanwhile river sand was treated ten to fourteen days earlier with 5 per cent ethyl mercuric iodide² at the rate of ½ gram per square foot (Lammerts, 1940). The seedlings were removed from the bottles with forceps, separated from the agar, and planted in 1½-inch thumb pots. These were then plunged in moist peat in flats and protected the first few days by inverting a wet flat over them, raising one end to a low angle.

In about three more weeks the seedlings were transplanted to three-inch pots. A compost of ½ loam,

²Obtained from Du Bay Chemical Company under the name of Du Bay 1155 HH.

⅓ sand, and ⅓ peat, which had been treated one week earlier with 5 per cent ethyl mercuric iodide at the rate of 1½ grams per square foot, was used. The seedlings were grown in these pots until the primary bark showed through in cracks at the base of the stem. They were then gradually hardened off by decreasing soil moisture and lowering the night temperature to 45° F. By November 1 growth had stopped, and shoots were sufficiently hardened to place the seedlings in a dark cold storage room at 40° F. After three to six weeks, depending on the type of hybrid, all leaves dropped off and plants were completely dormant. When they were removed to the greenhouse, growth was rapidly resumed and in a few weeks a shift to four-inch pots was necessary. By using a balanced nutrient solution the seedlings were grown to a height of 3 to 4 ft. in these pots. After hardening off in the lathhouse they were transplanted to the field in April or May during cloudy weather so as to avoid burning of the tender leaves. The seedlings were placed 4 ft. apart in the rows and the rows 4 ft. apart to allow for proper cultivation. They were furrow irrigated once per week, and liberally fertilized with ammonium phosphate (16–20) in July.

GERMINATION RESULTS.—Cross pollination of peach and nectarine varieties was begun in the spring of 1936. Approximately half of the seeds obtained from crosses involving the early mid-season peach varieties, Babcock and Early Imperial, as female parents were embryo cultured about three weeks after the fruit was ripe, that is about August 1, 1936. The remaining seeds from these crosses were stratified at 40° F. in flats of moist sand for three months. Of the 124 seeds from the Babcock crosses, 121 (or 98 per cent) germinated and grew into trees following embryo culture. Of the 111 stratified seeds, 97 (or 88 per cent) eventually germinated and survived. In the case of the Early Imperial crosses 189 of the 200 embryo cultured seeds, or 94 per cent, germinated and survived, as compared with only 57 of the 124 stratified seeds, or 46 per cent. Furthermore, many seedlings from both the Babcock and Early Imperial crosses did not emerge following stratification until April, and some did not come up until May, 1937. The germination results following stratification compare favorably with those reported by other investigators (Crocker and Barton, 1931) who reported an average of 41 per cent germination following stratification in peat at 5° C. for three months.

The seedlings from embryo-cultured and stratified seed obtained from these experiments were separately labeled and grown under as nearly comparable conditions as possible. They were lined out in May, 1937, and grown under comparable field conditions. Comparative data on the fruiting and flowering of these seedlings will be presented in a subsequent section.

In 1937, 45 different crosses of peach, apricot and nectarine varieties were made which involved 16 varieties as female and 21 varieties as male parents. About 10,500 flowers were emasculated and polli-

nated resulting in 2,536 fruits, an average of 24 per cent set. Only $1\frac{1}{2}$ per cent of the pollinated flowers set in the cross of Millers Late \times July Elberta³ and $8\frac{1}{2}$ per cent in the cross Sims \times July Elberta. In crosses such as these, and even for average cases, a high germination percentage is important, as large numbers of fruit can only be obtained by making many cross pollinations, an expensive and time consuming process. Accordingly all the seeds from the 1937 crosses were embryo cultured. Striking varietal differences in response of embryos to culturing were shown.

The embryos in 204 pits were so defective and shriveled as to be incapable of germination, while 15 pits had two kernels, thus making a net loss of 189. The completely shriveled embryos were mostly found in the following crosses:

	Pits with aborted embryos	Total pits
Mayflower \times July Elberta.....	112	150
Sims \times Transvaal Cling.....	10	175
Early Imperial \times Vainqueur..	11	81
Early Imperial \times July Elberta	7	102

Both Mayflower and Early Imperial are early types and characteristically have abortive embryos. Sims usually has a small percentage of fruit with split pits containing aborted embryos.

Of the remaining 2,347 embryos cultured, 593 failed to germinate. These with the exception of Mayflower \times July Elberta looked completely normal when cultured. In fact many partially shriveled embryos which seemed non-viable, germinated readily. The failure of the normal appearing embryos is most puzzling and merits careful study. They were found mostly in crosses involving Babcock, Lukens Honey, Early Imperial, and Rio Oso Gem as female parents. An average of 40 per cent of the embryos from these varieties failed to germinate. The percentages varied from 82 per cent failure in the case of the 1937 crosses of Babcock \times Vainqueur to 19 per cent in Rio Oso Gem \times Vainqueur. There was no relationship between the degree of earliness of the female parent and the percentage of failure. The 1938 group of embryos from Babcock \times Vainqueur germinated much better than the ones in 1937, possibly because they were cultured immediately after harvesting. This suggests that seed dormancy had begun in 1937 and that by culturing before ripening of the fruit, some of these embryos could be germinated. It is in line with this idea that the cotyledons of many of these embryos spread at right angles to the central axis and turned green. In some cases a slight epicotyl growth began. In recent experiments a few of these partially developed embryos were placed in cold storage at 40°F. for six weeks while still in the culture bottles. Upon removal to the

³ The female parent is given first in all crosses discussed in paper or listed in tables.

greenhouse some of them grew into normal seedlings.

Seven of the crosses made in 1937 involved nectarines both as male and female parents and in all cases the germination was high, averaging 82 per cent, as compared with an average of 69 per cent for the entire group of pits. The peach \times nectarine seedlings were above average in vigor.

In the spring of 1938, 1,228 cross pollinations were made resulting in 658 fruit, a 53 per cent set. This high percentage of set is due to the use of Babcock or its hybrids in most of the pollinations (table 1). The Babcock peach sets a high percentage of blossoms per tree as compared with varieties such as Sims, Lukens Honey, or Rio Oso Gem, and transmits this tendency to many of its hybrids. As will be shown later (table 3) the numbered seedlings listed in table 1 were from the 1936 hybrid seed embryo cultured August 1, 1936. It may thus be readily seen that many hybrid trees from crosses made in the spring of 1936 were used either as male or female parents or selfed in the spring of 1938.

The nectarine and double flowering characters in the peach are due to recessive factors. As both nectarines and double flowering peaches set very few fruit, it is necessary to use the F_1 hybrids of commercial peaches with nectarines and double flowering peaches as female parents when backcrossing. Accordingly, the fact that trees from embryo-cultured seeds are large enough to be used effectively as female parents two years after making original crosses is particularly important as a means of shortening the time involved in breeding new varieties of nectarines and double flowering peaches.

In 1939 approximately 2,900 self and backcross pollinations involving 32 selected hybrids from 1936 and 1937 crosses were made. These resulted in 1,114 fruit, a 39 per cent set. A total of 1,092 embryos were obtained from these fruit of which 900 or 81 per cent were successfully cultured. Nine of the hybrids involved nectarines, and it was again noted as was found in 1937 and 1938, that a high percentage of seeds from F_1 peach \times nectarine hybrids germinated and grew into very vigorous seedlings.

In the fall of 1940 an attempt was made to find a method of germinating seeds as rapid and efficient as embryo culture but involving less effort. Since only five weeks of growing time are lost in order to break dormancy of embryo-cultured seedlings and induce them to resume growth, any other method equally efficient can only allow for a similar period of time for cold storage treatment. Accordingly half the pits from eight different F_1 hybrid trees were stratified beginning August 30, 1940, for five weeks in moist sand at 40°F. They were then cracked and the kernels planted in small pots of treated soil which were placed in the greenhouses. The remaining half of the pits were cracked and embryo cultured on August 30, 1940. Although in one case 60 per cent germination was obtained following stratification and pericarp removal, the average germination was only about half that obtained following embryo culture (table 2). In some cases germination was very poor and the

TABLE 1. *Record of backcross, self and cross pollinations, fruit harvested, embryos cultured, and percentage germination in 1938 season. Crosses arranged according to type of backcross, self, or cross pollination.*

Type of backcross, self or cross pollination	Number of crosses or selfs	Number of fruit harvested	Number of embryos cultured	Number germinated	Per cent germinated
1. Backcrosses of Babcock to F ₁ (Babcock × Early Double Flowering Red and Pink):					
Babcock × 36057/4 ^a	100	51	49	27	53
Babcock × 36057/14	100	65	65	24	53
Babcock × 36057/17	100	64	63	52	80
Babcock × 36058/1	100	66	63	42	64
Babcock × 36058/15	120	50	47	33	66
Babcock × 36058/20	100	61	59	42	70
2. Backcrosses of F ₁ (Babcock × Coolidge Early Double Flowering Red) to Coolidge Early Double Flowering Red:					
36057/4 × Coolidge Early Double Flowering Red	75	46	46	40	87
36057/19 × Coolidge Early Double Flowering Red	75	33	32	26	79
3. F ₁ (Babcock × Early Double Flowering Red and Pink) Selfed:					
36057/4	75	30	28	26	86
36057/14	45	29	29	26	90
36057/19	12	8	8	8	100
36057/22	27	11	11	10	90
36057/23	45	17	17	17	100
36057/30	17	9	9	9	100
36057/42	18	6	6	6	100
36058/2	15	7	7	7	100
36058/16	30	21	21	14	66
36058/33	20	9	9	6	66
36058/35	9	7	7	5	70
4. Backcrosses of F ₁ (Peach × Nectarine) to Nectarine	50	17	17	15	88
5. F ₁ (Double Flowering × Early Imperial) × Barnes July Elberta	95	51	45	35	69
Totals	1,228	658	638	470	Ave. % 76

^a An understanding of the seedling numbers listed in this table is necessary in order to understand the significance of the data. Thus in group 2, the backcross 36057/4 × Early Double Flowering Red indicates that seedling 4 in population 57 obtained by crossing Babcock with the Early Double Flowering Red peach in the spring of 1936 was used as a female parent in the spring of 1938.

TABLE 2. *Comparative germination of peach seeds, embryo-cultured and stratified August 30, 1940. The pits were stratified in sand for five weeks at 40°F., pericarps then removed, and kernels planted in pots of treated soil. The records were completed December 6, 1940.^a*

Parentage of seed	Number of pits stratified	Number germinated	Per cent germination	Number of embryos cultured	Number germinated	Per cent germinated
F ₁ (Babcock × Quetta nectarine) open pollinated	86	38	44	85	57	67
F ₁ (Babcock × Boston) open pollinated	15	4	26	14	14	100
F ₁ (Goldmine × July Elberta) open pollinated	93	47	50	91	73	80
F ₁ (Lukens Honey × Boston) open pollinated	17	4	23	20	19	95
Totals	211	93	44	210	163	78

^a The records were completed by H. C. Swim of the Armstrong Nurseries, whose help and cooperation is gratefully acknowledged.

TABLE 3. *Comparative flowering and fruiting of peach trees from embryo-cultured and stratified seeds. Cross pollinations resulting in these trees were made in the spring of 1936 and records were taken in the spring and summer of 1938.*

Peach varieties used in crosses	Trees from seeds embryo cultured Aug. 1, 1936			Trees from seeds stratified Aug. 1, 1936, for 3 months at 40° F. in moist sand		
	Number flowering	Number fruiting	Total	Number flowering	Number fruiting	Total
36055—Babcock × Peppermint Flowering.....	12 ^a	..	13	11	..	24
36056—Babcock × July Elberta.....	25	9	25	4	4	17
36057—Babcock × Coolidge Early Double Flow- ing Red	30	30	30	16	16	17
36058—Babcock × Early Double Flowering Pink	23	22	23	11	11	12
36059—Early Imperial × Lukens Honey.....	23	10	32	23	5	47
Totals	113	71	123	65	36	117
Percentages	91	57		55	30	

^a There were a greater number of flowers per tree in the embryo-cultured group than in the control group from stratified seeds.

few seedlings which did emerge by December 6, 1940, were weak and slow growing. Obviously five weeks is not sufficient time for proper chilling of pits, though only three to five weeks is sufficient to break dormancy of seedlings from non-after-ripened seeds.

COMPARATIVE BEHAVIOR OF TREES FROM STRATIFIED AND EMBRYO CULTURED SEEDS.—The most outstanding results were those obtained with apricots. This species seems particularly well adapted to embryo culture technique, both as regards high percentage of germination and rapid vigorous seedling growth. By October 1, 1937, plants from embryos cultured in June were 1½ ft. high and ready for cold storage treatment at 40° F. Six weeks' storage was sufficient to satisfy their chilling requirements, for upon removal to the greenhouse they rapidly started growing again and continued growth until November, 1938. Many of these trees flowered and bore fruit in the spring of 1940, thus establishing a three-year breeding cycle for the apricot.

As shown in table 3, 91 per cent of the peach trees from seeds cultured August 1, 1936, flowered in the spring of 1938, two years from the time of hybridizing, while only 55 per cent of the trees from stratified seeds flowered. Almost twice as many trees in the embryo-cultured group bore fruit. Even more important from the breeding point of view was the greater number of flowers per tree. Accordingly many more fruit were set. Thus in the cross of Babcock × Early Double Flowering Red the average was 79 fruit per tree in the embryo-cultured group as compared with only 37 fruit per tree in the group from stratified seeds. For Babcock × Early Double Flowering Pink the ratio was 31 to 13. Several trees in the embryo-cultured group bore over 170 fruit and six others bore more than 100 fruit per tree. These trees, then, were satisfactory for selfing and as female parents in backcrossing as shown by results presented in table 1. Often 100 per cent of the selfed and hybrid seed from these and other two-year-old embryo-cul-

tured trees were germinated, thus supplying genetically random samples.

DISCUSSION.—Since embryo culture is a somewhat expensive method of germinating seeds, it is of interest to consider to what extent simpler methods may be used without decreasing the efficiency of the breeding program. Crocker and Barton (1931) and Flemion (1936) report from 27 to 87 per cent germination of varieties such as Elberta following pericarp removal and stratification of kernels in moist peat at 40° F. for ten to fourteen weeks. These germination results are comparable to those obtained by embryo culture. However, a period of six weeks to three months elapses before the kernels complete germination following stratification. Thus, in many cases six months of growing time are lost, and the young trees do not grow large enough to form flower buds by the following fall. A two-year breeding cycle is thus not possible. However, where rapid life cycles are not genetically important, removal of pericarp and stratification at 40° F. for ten to fourteen weeks is the simplest and least expensive method of germinating peach and other deciduous seeds.

Flemion (1934) has also found that if both pericarp and inner seed coats are removed and the seeds mixed with moist peat moss and kept at 25° F., germination occurs in five to seven days. From 37 to 94 per cent germination was obtained by this method, which is of course a simplified type of embryo culture, involving much less effort than when sterilized nutrient agar is used. The resulting plants are dwarfed in growth character, and require a period of three to five weeks of chilling before normal growth is resumed. It is important, as noted by Flemion, that injury to embryos be avoided, for fungi quickly attack the injured areas, decay sets in, and the embryos die. It is very difficult to avoid slight injuries, and so it is doubtful if high percentages of germination, especially of slow or weak embryos, can consistently be obtained by this method. In cases where hybrid

seeds may be fairly easily obtained and very high germination percentages are not genetically necessary, this method is very useful, inexpensive, and fully as rapid as the nutrient agar method of embryo culture.

It is quite important, when heterozygous varieties such as Babcock are used, that every potentially viable seed be germinated, for weak embryonic types may have characteristics of great horticultural value when matured into trees. Furthermore, a sound genetic analysis of horticulturally valuable types is dependent on securing random samples, which in turn depends on high or at least representative germination percentages. An interesting horticultural example is that of the saucer peach reported by Lesley (1939). Here the germination of saucer seeds was so low following ordinary stratification methods that the dominance of the saucer character was not at first suspected.

The higher percentage of embryo-cultured trees in bloom, as well as the greater abundance of flowers and heavier set of fruit, is not merely due to increased size of the tree, for some of the control trees from stratified seeds were fully as large as those from embryo culture. Even these, however, had very few flowers as compared with similar sized embryo-cultured trees. Evidently the application of cold treatment to satisfy the chilling requirements of seedlings from non-after-ripened seeds in some way causes the seedling trees to flower and fruit earlier and more abundantly than the same or even twice as much cold treatment applied to dormant seeds.

A further indirect advantage made possible by embryo culture is the saving in time and space needed for determining tree and fruit characters. As was mentioned above seedling trees were lined out four feet apart in the row and rows spaced four feet apart. This compact planting plan was possible because readings could be made the first spring after lining out on chilling requirements of the trees and later in the summer on such fruit characters as adherence of flesh to pit, color of skin and flesh, and shape of fruit. Accordingly, most of the trees could be re-

moved by the end of the second year, thus allowing ample room for growth of remaining seedlings and final selection three years after embryo culturing of the seeds.

SUMMARY

Embryo culture, as compared with usual stratification methods, results in a much higher percentage of germination in most apricot and peach crosses, thus giving a better random sample of genetic diversity.

Intermediate early types such as Early Imperial and hybrids ripening from the end of June until August characteristically have many abortive embryos which do not respond to stratification. These can be successfully germinated by embryo-culture methods.

In some crosses a large percentage of normal looking embryos was found which would not respond to embryo culture. The occurrence of these is not related to earliness of female or pollen parent. Some of these embryos can be germinated if given a cold storage treatment of six weeks at 40°F. while still in culture bottles.

Hybrid trees can be brought into flower two years from the time of cross pollination. Self pollinations and both male and female backcrosses can then be made every two years. These trees come into bearing two years from the time of harvesting the hybrid fruit, thus making it possible to take readings on such fruit characters as adherence of flesh to pit, skin and flesh color, and chilling requirements. Removal of undesirable seedlings by the end of the first year in the field row is thus possible.

An important saving of space is effected because the early removal of many seedlings allows the use of a four-foot square planting plan, without crowding of final tree selections.

Certain modifications in embryo-culture technique which have been found useful in avoiding failures are presented.

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A NEW SPECIES AND VARIETY OF *SCLEROCACTUS* FROM ARIZONA¹

Elzada U. Clover

SCLEROCACTUS havasupaiensis sp. nov. Simplex cylindricus erectus vel curvatus, 1.5–4.2 dm. altus, 8–11. cm. crassus; areolis subrotundis; costis 13–18, tuberculis prominentibus, spinis lateralibus 6–13, ra-

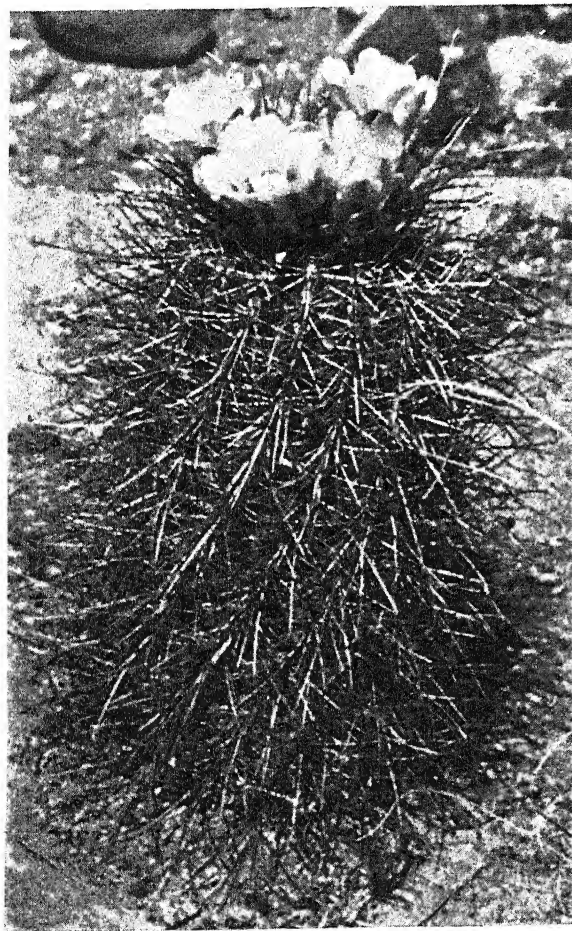


Fig. 1. *Sclerocactus havasupaiensis* Clover, sp. nov. Actual type specimen photographed in natural habitat at Havasupai Canyon, Arizona (Clover 5229 in part: there are several cotypes).

diatis, flexuosis, albidis, apice adustis, 10–20 mm. longis; centralibus 4–9, flexuosis, angulatis, curvatis, summis 3 compressis, pellucidis, ascendentibus, curvatis, 4–4½ cm. longis; inferioribus angulatis, purpureo-fuscis, 6–7 cm. longis; floribus in axillis summis, numerosis (vel usque ad 30), 3–5½ cm. longis, 3 cm. crassis, 5-seriatis; segmentis 2¾–3 cm. longis, apice obtusis, brevi-apiculatis, crassis; exterioribus in media parte atro-purpureis, margine albis; interioribus albis sed deorsum viridiusculi-luteolis visis; stylo viridi-luteo, 2 mm. crasso, glabro, stigmati-

lobis viridi-luteis, 6–8, deliquescentibus; filamentis numerosis, delicatis, viridi-luteis, antheris lutei-aureis, stylo brevioribus; fructibus carneis, purpureis, sed postea siccantibus. Specimen typicum siccatum ex loco dicto "Havasupai Canyon," Arizona, conservatum est in Herbario Universitatis Michiganensis (Clover 6404). Specimen vivum est in Horto Botanico Universitatis Michiganensis, Ann Arbor, Michigan.

Plant solitary, reaching a height of 42 cm., diameter 8–11 cm.; ribs 13–18 often spiraled, prominent, usually 1 cm. high, more or less tubercled; areoles subcircular, 7–12 mm. apart and alternating with those of adjacent ribs; glands in groove above young areoles well developed, 1–3 or sometimes as many as 6, yellowish to red, exuding considerable sweet, clear nectar; young seedlings 1 cm. in diameter bearing both radial and well-developed central spines; radial spines on mature plants 6–13, usually about 10, white with dark tips, flexuous, terete to flattened, acicular, 10–20 mm. long, lateral radials usually longer than upper and lower ones; central spines various, 4–9, often with upper ones ascending, straight, flattened and translucent, 4–4½ cm. long, sometimes resembling radials, lower centrals curved or hooked, flexuous, angled, extending variously, the longest 6–7 cm., always hooked and usually pointing downward, reddish, variegated red and white to



Fig. 2. *Sclerocactus havasupaiensis* Clover sp. nov. Two specimens in natural habitat on top of Supai sandstone formation showing association with *Agave utahensis*, *Rhus trilobata*, *Coleogyne ramosissima*, *Ephedra* and certain grasses.

almost black; flowers campanulate, appearing near the apex in upper part of the areole, as many as 30 buds and flowers at one time; flowers 3–5½ cm. long, 3 cm. across; perianth segments in 5 whorls, the two outer with segments short and obtuse, margins whitish with purple-drab shading to greenish up trace, inner segments entire, yellowish-green at base fading to almost white toward tip, 2–3 cm. long, 1½ cm.

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broad below the middle; scales on the ovary few, conic with rounded base, scarious, brown up trace, deciduous, 2 mm. long and containing sparse wool in axil; filaments extremely delicate, pale green, anthers bright orange, stamens shorter than the style, style 2 mm. in diameter, pale yellowish-green, stigma lobes 6-8, about the same color as style, glabrous, deliquescent, lobes appearing paired when immature, $3\frac{1}{2}$ mm. long, blunt-tipped; flowers remaining open several days, faintly scented; fruit about 1 cm. long, oblong, turning purplish but drying later; seeds shiny black, tuberculate, $2\frac{1}{2}$ -3 mm. long, much larger at upper end, hilum lateral, large, subcircular, much depressed. The type specimen (fig. 1) was collected by William Belknap, Jr., April 26, 1941, on top of the Supai Formation in Havasupai Canyon, Arizona, where the species grows abundantly. Specimens not in flower were previously collected by the author in July, 1940, in Havasupai Canyon (Clover 5229) near Navajo Falls, and in Hualapai Canyon (Clover 5100) on talus of the Supai Formation. A few plants were seen on the floor of Cataract Canyon above Supai, September, 1941.

This cactus more closely resembles *S. parviflorus* Clover than other species, but differs from it in several respects. The general appearance is less shaggy, and the central spines are more delicate and less flattened. Of the radial spines the lower ones are the longest in *S. parviflorus*. This new species usually has the lateral radial spines longer than the lower ones. Inner perianth segments of the Colorado River species are phlox purple; style and stigma lobes are also purple. The Supai species has whitish inner segments shading to yellowish-green below. Style and stigma lobes are pale yellow-green.

SCLEROCACTUS HAVASUPAIENSIS Clover var. **roseus** var. nov. Floribus roseis, suaveolentibus; segmentis perianthii oblongis, acuminatis; stylo viridi-luteo, 1 mm. crasso, glabro, stigmatibus purpureo-rubris, acuminatis. Specimen typicum legit E. Clover & Wm. Belknap, Jr. (Clover 6403), in Havasupai Canyon, Arizona, siccatum est in Herbario, Universitatis Michiganensis.

Similar in habit to typical *S. havasupaiensis*; flowers pale rose-pink with inner segments shading to pale yellow-green at base, decidedly sweet-fragrant; inner segments oblong, tips acuminate; style pale yellowish-green, stigma lobes reddish-purple, tapering toward the tip, not appearing paired when immature, not deliquescent; seeds with prominent tubercles. This variety was found in association with *S. havasupaiensis* on top of the Supai Formation in

Havasupai Canyon, Arizona, April 26, 1941. Young seedlings were growing at the base of the plants. Additional plants were collected by C. F. Shaffer, Jr.,

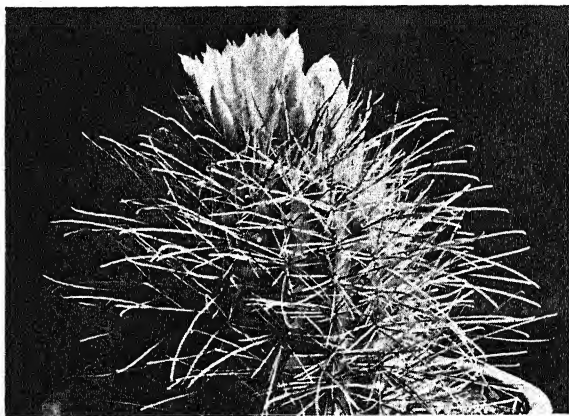


Fig. 3-4. *Sclerocactus havasupaiensis* var. *roseus* var. nov. Actual type specimen (Clover 6403) photographed while alive at Botanical Garden, University of Michigan, now dried and preserved as a herbarium specimen. Additional plants of the same collection (cotypes) are in the living collection (Bot. Gard. no. 18079).

in the same location in May, 1941, and are in the living collection (no. 18079) in the Botanical Gardens, University of Michigan.

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A NEW FUNGUS BELONGING TO THE CLADOCHYTRIACEAE¹

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AS RECENTLY summarized by Berdan (1941), the family Cladochytriaceae includes eight well recognized genera. The sporangia of six of these genera dehisce following the gelatinization of the tip of the exit papillae, while the sporangia of the other two genera are operculate.

In the inoperculate group are included the genera *Cladochytrium* (Nowakowskii, 1877), *Amoebochytrium* (Zopf, 1884), *Physocladia* (Sparrow, 1932), *Catenaria* (Sorokin, 1876), *Mitochytridium* (Dangeard, 1911), and *Physoderma* (Wallroth, 1833). The thalli of species of *Urophlyctis* (Schroeter, 1889) and of *Physoderma* are so much alike that most critical students of the family are more or less agreed that at present the criteria for separating these genera are superficial and inadequate. In the operculate group there are two genera, *Nowakowskiella* (Schroeter, 1897) with three species, and *Septochytrium* (Berdan, 1939), a monotypic genus. The species of *Nowakowskiella* at present accepted are: *N. elegans* (Schroeter, 1897), *N. ramosum* (Butler, 1907), and *N. profusum* (Karling, 1941a). The chytrid described as *Nowakowskiella endogena* Constantineanu by Domjan (1935) is probably the same as the species first described from India by Butler (1907) and just recently reported from Texas by Karling (1941b). The present paper reports a new species belonging to the Cladochytriaceae which is tentatively being placed in the genus *Nowakowskiella*, although the type of resting spore formation in this new species is fundamentally different from that of any of the other species of the genus.

During the course of my examination of various types of bait that had been placed in water samples collected from pools and streams of eastern Illinois, I have encountered numerous chytridiaceous fungi, one of which is of particular interest. This chytrid was first isolated on cellophane as recommended by Haskins (1939). The water samples from which it was obtained were taken near Penfield, Illinois, in the fall of 1940. It was collected again during the early summer of 1941, from Crystal Lake, Urbana, Illinois, likewise growing on cellophane bait that had been placed in a water sample. In addition to cellophane as a substratum, other materials have been used with success. Growth of the fungus on portions of grass leaves or corn seedling leaves, and on filter paper or lens paper as recommended by Couch (1939) has been quite extensive. Growth on plain agar is sparse.

Unifungal cultures of this chytrid have been maintained on various types of substratum for over nine months, and observations indicate that growth on all is more or less uniform. The principal differences are produced by growth on cellophane where the cultures are less extensive, more compactly developed, have more swellings, and a larger percentage of inter-

calary sporangia and resting bodies. The following description of this species is based primarily on cultures growing on sterilized corn seedling leaves.

The resting bodies of this fungus, particularly those with more than one resting spore, bear a superficial resemblance to oogonia containing mature oospores, such as are found in certain other aquatic Phycomycetes. Resting spores are in no sense oospores, as is seen from their developmental stages, and their true nature is discussed in another part of this paper. The majority of resting bodies contain but a single resting spore which is somewhat flattened on one side so that it is more or less hemispherical in shape. Therefore, the name *Nowakowskiella hemisphaerospora* is proposed for this new species.

Nowakowskiella hemisphaerospora sp. nov.—Rhizomycelium much branched, extensive, hyaline, filaments quite variable in diameter, spindle shaped or oval swellings numerous or very much scattered. Zoosporangia usually terminal on short lateral branches or occasionally intercalary, smooth, hyaline, operculate, quite variable in shape and size but commonly ovoid, ellipsoid, or pyriform, usually $7.5\text{--}14.2\ \mu \times 9.5\text{--}28.4\ \mu$ (commonly $11.5 \times 17.2\ \mu$) with one to several exit papillae (or tubes), apical, subapical, or lateral, varying in length up to $18\ \mu$ and usually about $4.0\text{--}4.7\ \mu$ in diameter; operculum circular, $3.1\text{--}3.5\ \mu$ in diameter, either remaining attached to the sporangium or lying loose nearby. Zoospores hyaline, spherical or ovoid, $4.4\text{--}6.3\ \mu$ in diameter, with a single refractive globule; cilium attached posteriorly, $32.5\text{--}40.0\ \mu$ in length. Resting bodies terminal or intercalary, usually somewhat ellipsoidal, containing one to four thick-walled hyaline resting spores and a corresponding number of empty cells. Resting spores rather uniform in size, $8.5\text{--}12.6\ \mu \times 11.6\text{--}15.6\ \mu$ (commonly $11.0 \times 14.2\ \mu$) usually somewhat hemispherical in shape; refractive globule in mature resting spores large with several smaller ones commonly surrounding it. Germination unknown.

Nowakowskiella hemisphaerospora sp. nov.—Rhizomycelium copiose ramoso, extenso, hyalino, filamentis diametris variis, incrementis fusiformis aut ovatis, multis vel permultis dissipariis. Zoosporangiis plerumque terminalibus in ramo lateralis vel nonnumquam intercalaribus, levibus, hyalinis, operculatis, forma atque magnitudo vario, saepe ovoideis, ellipsoideis, aut pyriformibus, plerumque $7.5\text{--}14.2\ \mu \times 9.5\text{--}28.4\ \mu$ (plerumque $11.5 \times 17.2\ \mu$), cum papillis exeuntibus (vel tubulibus) una vel compluribus, apice aut sub apice aut a latere formato, variatis longitudine usque ad $18\ \mu$, atque plerumque $4.0\text{--}4.7\ \mu$ diametro; operculo orbiculato, $3.1\text{--}3.5\ \mu$ diametro, aut sporangio affixo aut liberato adiacente. Zoosporiis hyalinis, sphaericis vel ovoideis, $4.4\text{--}6.3\ \mu$ diametro, cum refractivo globulo singulo; cilio posteriore affixo, $32.5\text{--}40.0\ \mu$ longitudine. Truncis perdurantibus aut terminalibus aut intercalaribus, aliquid ellipsoideis,

¹ Received for publication July 23, 1941.

uno ad quattuor sporiis perdurantibus parietibus crassis, hyalinis, atque aequalis numerarum vacuum cellarum contineriis. Sporiis perdurantibus relativo constanti magnitudine, $8.5-12.6 \mu \times 11.6-15.6 \mu$ (plerumque $11.0 \times 14.2 \mu$) plerumque aliquantenus hemisphaericis, refractivis globulo magno in sporiis maturiis cum parvis globulis plerumque circumvaleris. Germinatione incompta.

spicuous refractive globule, typical of a chytrid zoospore. After an active swimming period these zoospores become rather sluggish, move about in an amoeboid fashion for a while, and finally settle down on the substratum to germinate. A single germ tube is commonly developed (fig. 4) which soon produces rhizoids and branches to form the rudiments of the rhizomycelium (fig. 5). Usually a spindle-shaped

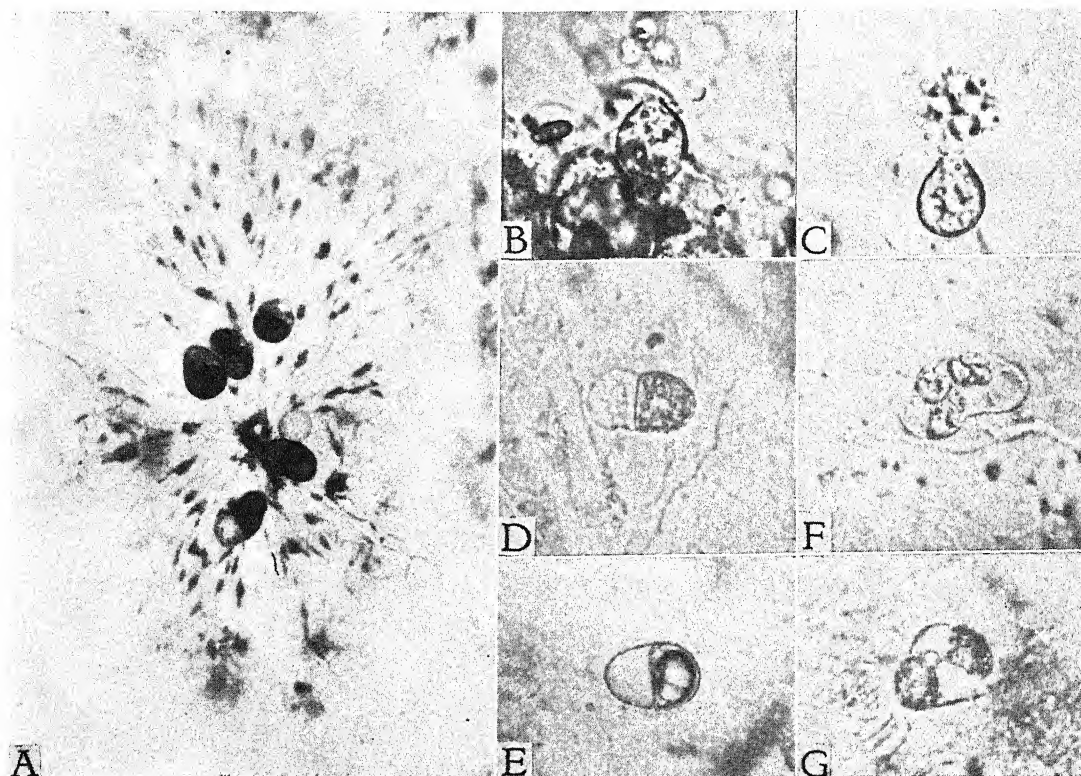


Fig. 1. Photomicrographs.—A. A young thallus growing on cellophane, showing five resting bodies, a single empty sporangium just to the right of center, and numerous swellings in the rhizomycelium. Stained with cotton blue.—B. Extramatrix sporangium after escape of most of the zoospores showing the operculum slightly to the right of the orifice.—C. Extramatrix sporangium from which spores are emerging showing the operculum at the margin of the orifice.—D. Developing resting body. Note protoplasm accumulating in one cell.—E. Resting body containing a single mature resting spore.—F. Resting body containing three developing resting spores.—G. Resting body containing two developing resting spores. Note that the number of empty cells in F and G corresponds to the number of developing resting spores. A $\times 380$, all others $\times 570$.

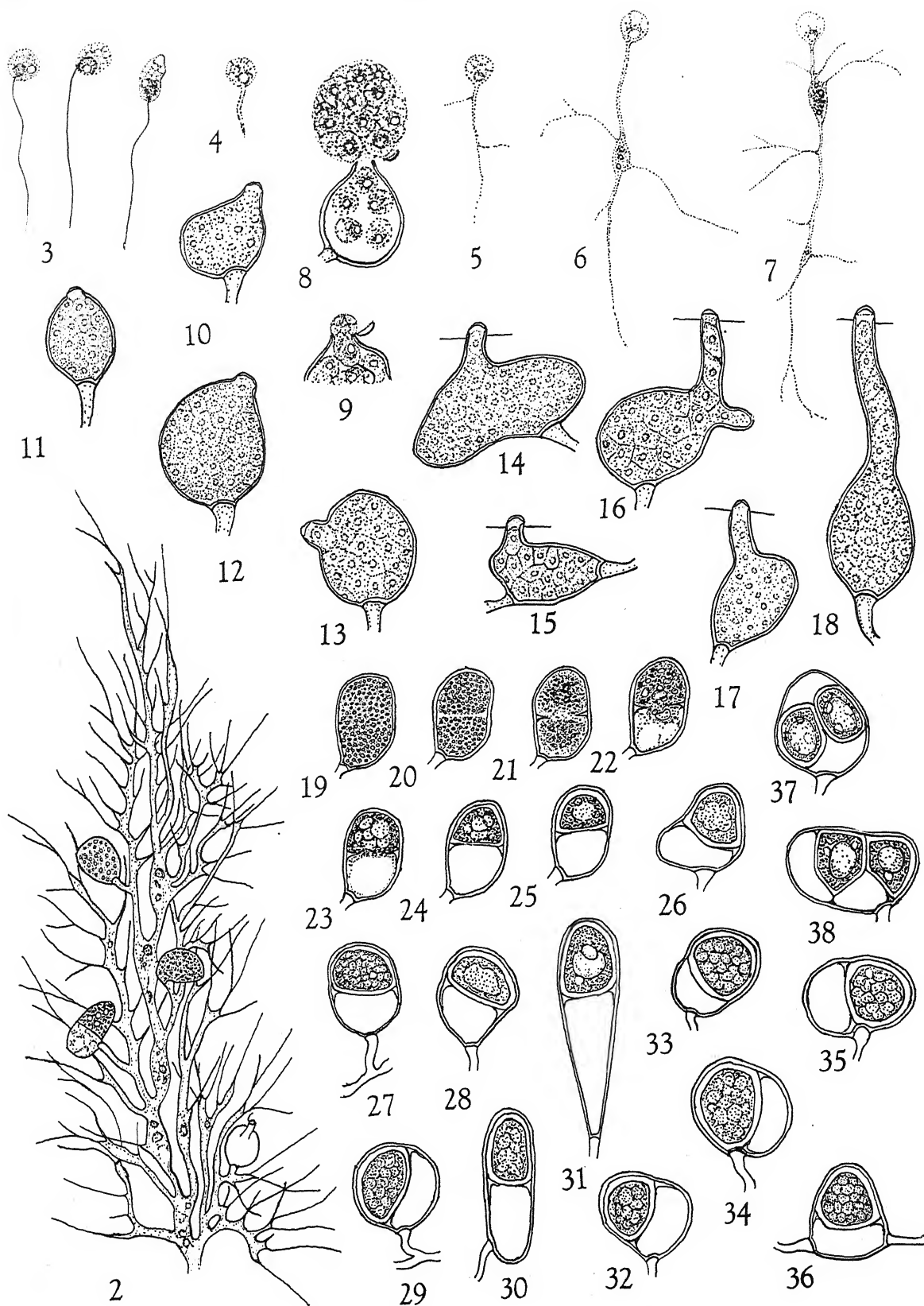
Collected as a saprophyte growing on cellophane placed in water samples taken near Penfield, Illinois, October 5, 1940, and isolated in the same manner from water samples from Crystal Lake, Urbana, Illinois, by the author, May 25, 1941, and from Dixon Spring, Illinois, by H. W. Larsh, June 12, 1941.

This fungus is quite distinct from any of the known Cladochytriaceae, and is easily distinguished from other species of *Nowakowskiella* by its characteristic resting bodies which always have been found in abundance.

DEVELOPMENT OF THE THALLUS.—The zoospores of *N. hemisphaerospora* (fig. 3) are characteristically globular in the swimming stage with a con-

swelling develops in the germ tube before much branching has taken place (fig. 6, 7). The nucleus from the spore migrates into this spindle swelling. This change in the position of the nucleus is easily demonstrated in cultures growing on cellophane by the addition of cotton blue to a preparation. In unstained material, several small refractive globules are prominent in each spindle-shaped swelling. In the filamentous rhizomycelium these refractive globules are absent or extremely small and the cytoplasm appears almost homogeneous, having a somewhat gleaming cast.

As the rhizomycelium becomes more extensive, the principal filaments become thicker with much irregularity in width and with many swellings in the older



portions (fig. 1A, 2). The swellings may be fusiform or more or less globose or elongate. Numerous rhizoids arise along the filaments and these terminate in very fine points. I have been unable to obtain even the slightest indication of a positive reaction for the presence of cellulose in the walls of any portion of the thallus with chloro-iodide of zinc.

The sporangia and resting spores most frequently arise at various places on the rhizomycelium on the ends of the short lateral branches (fig. 1, 2, 8, 10-14, 16-35, 37, 38). Intercalary sporangia (fig. 15) and resting bodies (fig. 36) have been observed frequently, but these are decidedly fewer in number than those produced terminally.

Sporangia formed extramatrically are, for the most part, pyriform, obpyriform or somewhat globose in shape with short exit papillae (fig. 1B, C; 8-13). There is ordinarily just a single papilla on extramatrical sporangia, and this is usually almost apical in position. Subapical and lateral papillae, however, are not uncommon.

Intramatrical sporangia (fig. 14-18) vary more in shape and size than do those produced extramatrically. Exit tubes are also much longer than the exit papillae of extramatrical sporangia. The fundaments of several exit tubes may be formed by a single sporangium or the single tube may have one or more prominent diverticulae which resemble papillae (fig. 16). As far as I have been able to observe, only one papilla is functional. The length of the exit tubes is determined by the depth at which sporangia are formed below the surface of the substratum. The tip of the tube never has been observed to protrude much above the substrate surface.

Sporangial proliferation occurs in *N. hemisphaerospora*, but is quite rare and not nearly as common as it is in species of *Cladochytrium* (Nowakowski, 1877; Karling, 1931; Berdan, 1941).

Stages in sporangial development of *N. hemisphaerospora* are identical in their main features with those of typical chytridiaceous fungi. These protoplasmic changes have been described by others (Berdan, Couch, Karling, Sparrow, etc.) for developing sporangia of several species of the Cladochytriaceae and of the Rhizidiaceae, so need not be repeated in detail here.

When sporangia of *N. hemisphaerospora* are mature and zoospores are discharged, the operculum is either carried away by the emerging spores (fig. 1B, 8) or is pushed to one side and remains attached to the margin of the orifice (fig. 1C, 9). The presence of an operculum on all sporangia was at first a point in question, for it was found attached to only a rather

small percentage of the empty sporangia. Observations on developmental and germination stages of many sporangia, however, revealed the fact that the operculum is frequently completely detached and is moved away some distance from the sporangium.

Here, as in other chytrids where the necks of the emergence tubes are rather narrow, the orifice through which the spores escape is smaller in diameter than are the normal spores. Therefore, as the spores escape from sporangia, they become elongate as they move into the exit tubes and emerge (fig. 9), but assume the globular shape soon thereafter (fig. 8). The zoospores of *N. hemisphaerospora* are held together by a hyaline matrix for a short while after emergence and are not enclosed in a vesicle as reported for *Physocladia* by Sparrow (1932).

RESTING SPORE FORMATION.—The resting bodies of *Nowakowskella hemisphaerospora* are formed by the thallus at the same time that zoosporangia are being produced or even before zoosporangial formation begins. Often very young thalli may have several developing resting bodies before any zoosporangia have reached maturity. However, as a thallus becomes more mature, the production of resting bodies far surpasses the formation of zoosporangia.

The majority of resting bodies arise as terminal enlargements of short lateral branches of the main filaments of the rhizomycelium, but some have an intercalary origin. In early stages of development, young resting bodies can hardly be distinguished by their shape and size from early stages in sporangium formation. The protoplasm of young resting bodies, however, is much more dense and contains many more minute refractive globules than do young sporangia.

With the exception of those that are of an intercalary origin, resting bodies arise in most cases without any definite relationship to the swellings of the rhizomycelium. Most of the resting bodies are of a rather uniform size and contain at maturity a single thick-walled resting spore (fig. 1E; 25-36). The larger resting bodies may contain as many as four resting spores. Resting spores, whether produced singly (fig. 25-36) or with others in a resting body (fig. 37, 38), are of a relatively uniform size. When more than one resting spore is formed in a resting body, the individual resting spores are slightly smaller than the average size of those formed singly. Within the walls of each resting body, accompanying the resting spores, are empty cells, thought to be "male" cells, whose protoplasm has previously united with that of each cell which matured to form resting spores.

Fig. 2-38.—Fig. 2. Portion of thallus growing on cellophane to show characteristics of rhizomycelium.—Fig. 3. Zoospores.—Fig. 4-7. Germinating zoospores in various stages of development.—Fig. 8. Zoosporangium showing spores being discharged into a matrix.—Fig. 9. Tip of sporangium showing emergence of a spore through the orifice of exit papilla.—Fig. 10-13. Extramatrical sporangia showing typical shapes and various positions of exit papillae.—Fig. 14-18. Intramatrical sporangia. Fig. 16 shows a diverticulum from the point of origin of the exit tube.—Fig. 19-25. Stages in formation of a resting spore.—Fig. 26-35. Various shaped resting bodies which contain single resting spores illustrating positions that resting spores may occupy within resting bodies.—Fig. 36. An intercalary resting body with a single resting spore.—Fig. 37-38. Resting bodies with two resting spores and two empty cells in each. (Fig. 2, $\times 438$; all others $\times 850$.)

Young resting bodies arising in a terminal position at first enlarge in a globose fashion but soon become elongate (fig. 19). Elongation very frequently takes place in such a manner that the long axis of the resting body is more or less at right angles to the stalk. The protoplasm that has been accumulating in the developing resting body is considerably more granular and more dense than in any other part of the thallus. Many small refractive globules also accumulate in these developing resting bodies.

As development progresses, a hyaline central region becomes evident in young resting bodies indicating that the protoplasm has undergone cleavage (fig. 20). The two parts thus formed are of approximately the same size. Between these two protoplasts an incomplete wall is deposited which separates them, except in the center where the wall is not deposited until later (fig. 21). During the formation of the wall, the refractive globules in each cell begin to coalesce and those in one cell become increasingly fewer (fig. 22). The movement of the protoplasm from one cell to the other is very gradual and takes at least twelve to fourteen hours; consequently, it is very difficult to observe the actual movement of the protoplasm. It can be readily observed, however, that the protoplasm in one cell becomes more vacuolate as the protoplasm in the other becomes more dense. The amount of refractive material also becomes more abundant in the cell in which the protoplasm is accumulating (fig. 22, 23). When practically all of the protoplasm has moved from one cell into the other, the cell that contains the dense protoplasm forms a thick wall. This wall is always fused with that of the resting body so that the spores within never lie entirely free. The refractive material in the thick-walled cell continues to coalesce until one large globule with several smaller ones can usually be observed in each mature resting spore (fig. 24, 25).

As far as can be observed in living material, the stages in development of the several-spored resting bodies of *N. hemisphaerospora* are essentially like those of resting bodies in which a single resting spore is formed. The fusion of the different pairs of cells in the developing several-spored resting bodies takes place more or less simultaneously and the resting spores reach maturity at approximately the same time. The germination of resting spores has not been observed.

As yet no detailed study has been made of the nuclear behavior in resting spore formation in *N. hemisphaerospora*, but observations on cultures growing on cellophane that have been stained with cotton blue indicate that a nuclear fusion may occur. By the time cleavage of the protoplasm within a resting body has taken place, each cell contains a single nucleus. Later stages in development have two nuclei in one cell and none in the other, while the mature resting spore appears to be uninucleate. The passage of nuclei from one cell to another or the actual fusion of the nuclei has not been observed in the material fixed and stained to date, but it appears that with proper diligence these stages could be

found. In resting bodies containing more than two cells, each cell is also uninucleate in early stages, and the same is true of the mature resting spores.

DISCUSSION.—It appears that the type of resting spore formation observed in *Nowakowskiella hemisphaerospora* is unique for the Cladochytriaceae. The cells that fuse are formed by the cleavage of one protoplast and are approximately equal in size. Although mature resting spores may resemble oospores, the fusion process is not oomycetous.

The hemispherical or flattened resting spores of species of *Urophlyctis* may appear similar in some respects to the resting spores of *N. hemisphaerospora*. Some authors have considered this characteristic shape of the resting spores of *Urophlyctis* of sufficient importance to warrant the retention of *Urophlyctis* as a genus distinct from *Physoderma*. The resting spores of *Urophlyctis* have been thought by some to develop following a conjugation of similar cells and Schroeter (1897) included this genus, along with certain other chytridiaceous fungi in which sexuality was known or suspected, in his newly created family, the Oochytriaceae. *Nowakowskiella hemisphaerospora* could not be included in the genus *Urophlyctis* in our present system of classification because of its operculate sporangia. In the Chytridiales, whether sporangia are operculate or inoperculate is a taxonomic character of fundamental importance.

Certain of the resting spores of *Nephrochytrium stellatum*, described and figured by Couch (1938), appear similar to the typical resting bodies of *Nowakowskiella hemisphaerospora*. His figures 25 to 29 also indicate a similar development to that of the single-spored resting bodies of *N. hemisphaerospora*, but nothing of the nuclear behavior is discussed. Couch points out that the resting bodies of *N. stellatum* resemble the azygospores of certain of the Mucorales. It might be noted here also that the azygospores of *Azygozygum chlamydosporum* Chesters (1933) rather superficially resemble the single-spored resting bodies of *Nowakowskiella hemisphaerospora*. The azygospores of *Azygozygum*, however, are multinucleate structures.

SUMMARY

A new species of the genus *Nowakowskiella* is described. It is characterized by a rather extensive rhizomycelium, operculate sporangia which lack an apophysis, and by resting bodies that contain one or more resting spores which are formed following the fusion of paired protoplasts. This fungus grows saprophytically on cellophane, on boiled grass or corn seedling leaves, and other similar substrata. It is distinguished from other species of the genus by the numerous resting bodies which, as a rule, contain a single hemispherical, hyaline, smooth-walled resting spore. Resting bodies, however, may contain up to four resting spores.

The cytology of resting spore formation has not

been studied in detail, but there is some evidence to indicate that a nuclear fusion may occur in the formation of the resting spores.

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A DEVELOPMENTAL ANALYSIS OF CELL LENGTH AS RELATED TO STEM LENGTH¹

Elizabeth A. Bindloss

INTEREST IN the problem of cell size and its relation to organ size in plants was first stimulated in the latter part of the nineteenth century by the investigations of Sachs (1893), Amelung (1893), Frank (1895), and Gauchery (1899). These investigators attempted to determine whether a positive correlation existed between the size of the organ and the size of its constituent cells at maturity. Such studies failed to recognize the changes in size relationships of cell and organ which occur during ontogeny. It is only within the past decade that the developmental aspects of the problem of cell and organ size have been considered.

The present investigation has had as its object the analysis of the changes in cell lengths in the embryonic region of the stem apex and their relation to stem length at successive stages of the plant's vegetative development. The study has been extended to include a comparison of cell lengths in genetically tall and dwarf varieties of the same plant species in order to determine whether a positive or negative correlation exists between plant height and the

length of the embryonic and elongating cells in the shoot apex. Such a developmental analysis of the growth of immature cells, on which genes presumably exert their greatest influence, should throw further light on the rôle played by those genetic factors which directly or indirectly control the ultimate size of cells and organs.

MATERIALS AND METHODS.—Good material for a study of cell-organ size relationships is found in the stem tip, where cells by their continual division and elongation give rise to the fundamental structure of the plant axis and its associated organs. Growth of the stem in most cases is strictly polar, and its cells are likewise polarized in their elongation parallel to the stem axis. Sierp (1913) in his studies of genetically tall and dwarf plants emphasized the importance of using cell length rather than cell width or volume in any investigation of the relationship of cell size to plant height. Thus in the present study cell length is used as a measure of cell size in analyzing differences in height between tall and dwarf plants in which height is controlled by genetic factors.

Preliminary investigations of genetically tall and dwarf varieties of *Antirrhinum majus*, *Phlox Drummondii*, *Salvia splendens*, *Ageratum Houstonianum*, *Verbena hybrida*, *Tagetes patula*, *Petunia hybrida*, *Lycopersicum esculentum*, *Zinnia elegans*, and *Pi-*

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sum sativum showed that certain of these plants were not favorable for a developmental study of cell size in relation to height. Suitable material was provided by *Lycopersicum esculentum* and *Zinnia elegans*, for in these plants the prominent main axis of the plant enabled overall height to be measured accurately, the plants grew rapidly, and the seed size was sufficiently large for the embryo to be removed *in toto* from the seed coat.

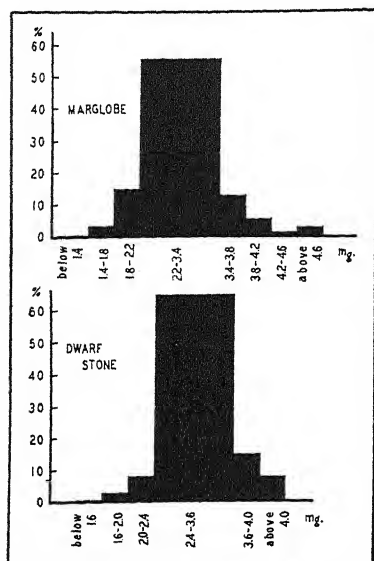


Fig. 1. Distribution of seed weights of Marglobe (tall) and Dwarf Stone tomatoes.

Two commercial strains of *Lycopersicum esculentum* were studied. Marglobe is a tall variety, homozygous for the height factor *D*. Dwarf Stone, homozygous for the dwarfing factor *d*, has the characteristics described for such tomato plants by MacArthur (1926). The stems are shortened and stocky; cotyledons, leaflets and petioles short and broad; leaves somewhat puckered and downcurved and foliage deeper green in color than that of the Marglobe variety. Leaf number in Marglobe and Dwarf Stone was the same in each of the stages studied, but stem diameter was greater in Dwarf Stone. Differences in stem length of the two varieties appeared as early as the cotyledonary stage.

The zinnia varieties Giant and Lilliput were studied. Giant normally grows to 2½ and 3 feet, while Lilliput grows to about 1 foot. Breeding behavior of these zinnias indicates that height is due to multiple factors (Burpee, 1940). The two varieties resemble each other closely in leaf size, shape, and number. At maturity, however, flower size was somewhat greater in the Giants than in Lilliputs. A greater mean seed weight (determined after removal of the pericarp) in Giant plants is associated with their larger flower size.

Because of conflicting reports concerning the effect of seed weight on plant height, it seemed advisable in a study of tallness and dwarfness to use

seeds of tall and dwarf plants which were essentially equal in weight. Therefore, all seeds were weighed singly on a Roller-Smith precision balance. Observed height differences then might be expected to be due to inherent differences in the plants' developmental patterns rather than to an advantage on the part of one plant brought about by greater "capital" in the embryo. Before weighing the zinnia achenes, the outer husk or pericarp was removed.

The distribution of tomato and zinnia seed weights is shown in figures 1 and 2. It was found that the modal class of the Marglobe seeds was from 2.2 to 3.4 mg.; and of the Dwarf Stone seeds from 2.4 to 3.6 mg. The seeds in these two groups were planted, for all practical purposes the difference in the two groups being so slight that they may be considered essentially equal. In zinnias, the modal class for Giant seeds was 4.0 to 5.5 mg.; and for Lilliputs 3.5 to 5.0 mg. These were the classes used. There was a much greater elimination of large and small seeds than in tomato as is indicated by figure 2.

All seeds were planted in flats of garden soil the first of July. The zinnias were transplanted into adjacent field plots fifteen days later; the tomatoes four weeks later. In so far as possible the tall and

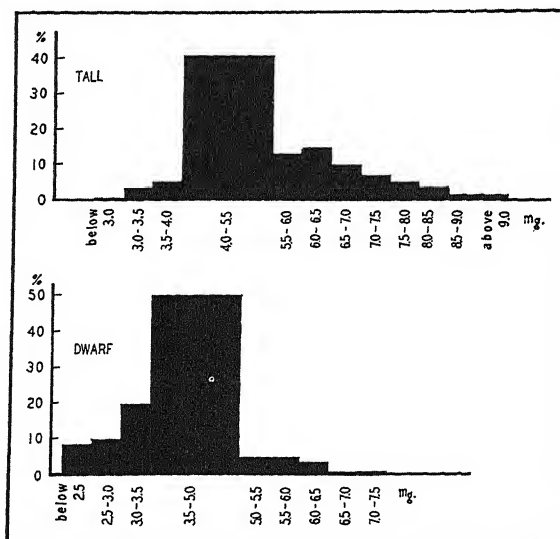


Fig. 2. Distribution of seed weights of Giant and Lilliput zinnias.

dwarf plants used in this study were grown under identical conditions. In the tomatoes, where the variability in height was slight, 100 plants were measured in the early stages of development, but fewer were measured at each successive stage as the number became reduced. The minimum number of tall or dwarf plants measured at any one time was 50. Because the zinnias were more variable in height in the early stages of growth, 150 plants were used in determining overall mean height. These measurements began when the hypocotyl was 3 to 5 mm. above the ground and were continued approximately every five days thereafter.

Of the stem tips collected for histological study, those representing four stages in the vegetative ontogeny of the plant were arbitrarily selected for further investigation. These stages for tomatoes were (I) the dormant embryo; (II) 7-day-old seedlings; (III) 23-day-old plants; and (IV) 38-day-old plants. Zinnia plants at stages comparable to II, III, and IV were 9, 15, and 22 days old. Stage III was selected as being intermediate in point of time between the seedlings and the oldest plants. Stage IV included plants selected five days before the first flower primordia were visible.

All stem tips were killed in CRAF solution, dehydrated by the butyl alcohol method, and embedded in paraffin. Longitudinal serial sections were cut at a thickness of 8μ . Five individual stem tips of both tall and dwarf forms were studied at each developmental stage mentioned above. On each slide bearing a single stem tip at least five sections were approximately median. In each of these five sections, cell lengths of a series of successive cells were measured, proceeding in a proximal direction from the stem apex (see fig. 3). Since there were five stem tips of each stage and separate determinations were made for five median sections of each, the mean length of each cell in the series was based on 25 measurements. Thus the mean length of the first cell at the apex was determined by 25 measurements, the second cell by 25, etc. Cell lengths were measured with an ocular micrometer (oil immersion objective) and converted to microns. A total of approximately 35,000 cells was measured.

Of the cells in the shoot apex, usually the first ten, forming the promeristem, were isodiametric and non-vacuolate. Cells beyond this region are in various stages of vacuolation. In the embryos and seedlings of both zinnias and tomatoes the 50th cell from the

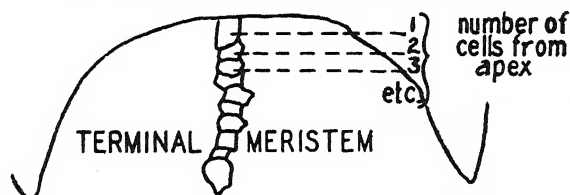


Fig. 3. Diagram of a typical terminal meristem showing a series of successive cells.

tip was relatively mature and had almost reached its maximum length. However, in later stages it was necessary to extend the region of cell measurement in order to find cells which were of constant length. All of the cells measured, with the possible exception of the older cells in the seedling hypocotyl, were embryonic in the sense that they were located in a region where cell division is still going on, *i.e.*, mitotic figures were discernible.

OBSERVATIONS.—HEIGHT OF TOMATOES AND ZINNIAS.—Marglobe and Dwarf Stone tomatoes showed visible height differences when the hypocotyl first appeared above the ground. The greater height of the Marglobe plants was maintained throughout all

later stages of development as is shown in figure 4. When plotted logarithmically the height measurements of the two varieties lay along two parallel straight lines. Thus the rate of stem elongation is the same for each variety.

The initial differences in hypocotyl lengths of the Marglobe and Dwarf Stone seedlings suggested that their height differences were already present in the

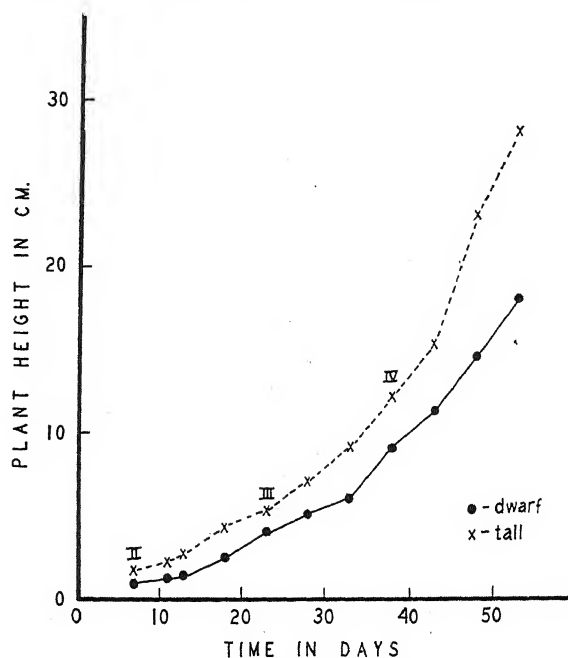


Fig. 4. Height of Marglobe and Dwarf Stone tomatoes grown in soil. Roman numerals show successive stages of development at which cell lengths were determined.

seed or established at some time between seed dormancy and the appearance of the seedling above the soil. Dormant embryos of the two varieties, dissected from seeds of the same weight, also showed differences in length: embryos of Marglobe tomatoes were 1 mm. longer than Dwarf Stone embryos. When the two kinds of seeds were germinated on filter paper the Marglobe hypocotyls were always longer than those of Dwarf Stone until growth ceased (fig. 5). Thus tallness or dwarfness in tomatoes is already established in the seed and is not solely a function of later development.

Although Giant and Lilliput zinnias ordinarily show a marked difference in height, under the conditions of the present investigation such plants exhibited little or no height differences during vegetative growth. When the flowers were fully formed, however, the Giant plants were taller than the Lilliputs. Figure 6 shows the height of zinnias at successive stages in development. Bailey (1939) stated that it was necessary to plant zinnias early in the season in the North in order to obtain specimen plants 3 feet tall. Since the zinnias of this study were not planted until July 1, it is possible that the tall zinnias, which

are especially sensitive to length of day, failed to develop to their fullest extent.

The weight of the seeds may be a more significant factor than the environment of these plants in deter-

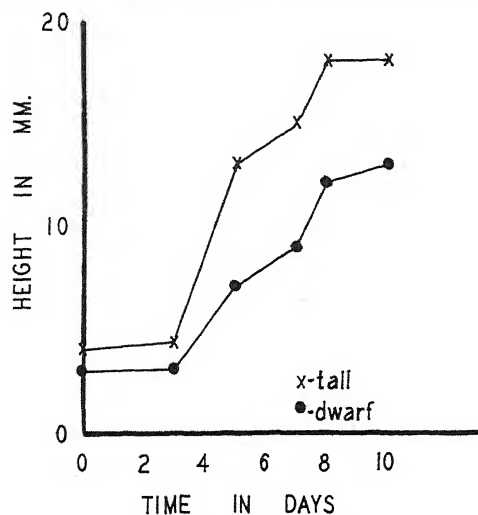


Fig. 5. Height of Marglobe (tall) and Dwarf Stone tomatoes grown on filter paper.

mining their height. The seeds of Giant and Lilliput zinnias, selected for planting, were of approximately uniform weight. However, in the entire sample 48.7 per cent of the Giant seeds were heavier than those in the modal class while only 13.5 per cent of Lilliput

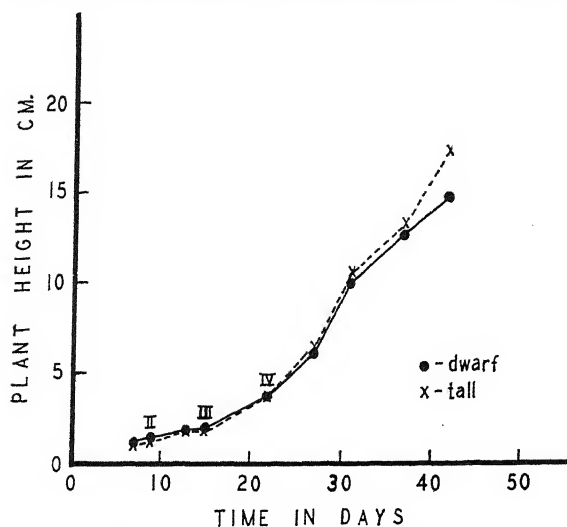


Fig. 6. Height of Giant and Lilliput zinnias grown in soil. Roman numerals show successive stages of development at which cell lengths were determined.

seeds were heavier. Conversely 35.2 per cent of Lilliput seeds were lighter than those of the modal class, whereas only 11.4 per cent of the Giant seeds weighed less. Thus in selecting seeds of similar weights, the relatively *large* Lilliput seeds were planted and the relatively *small* Giant seeds.

In the tomato varieties Marglobe and Dwarf Stone there was little difference in the normal distribution of the respective seed weights (fig. 1). Thus, although there may be a positive correlation between seed weight and plant height, it could not be demonstrated by the use of these tall and dwarf varieties. It seems probable that such a correlation may exist in zinnias and that the relatively heavy Giant seeds ultimately produce taller plants than do Lilliput seeds. If this is true, then the selection of seeds of nearly uniform weight would result in the similar heights of both "tall" and "dwarf" plants as observed in this investigation.

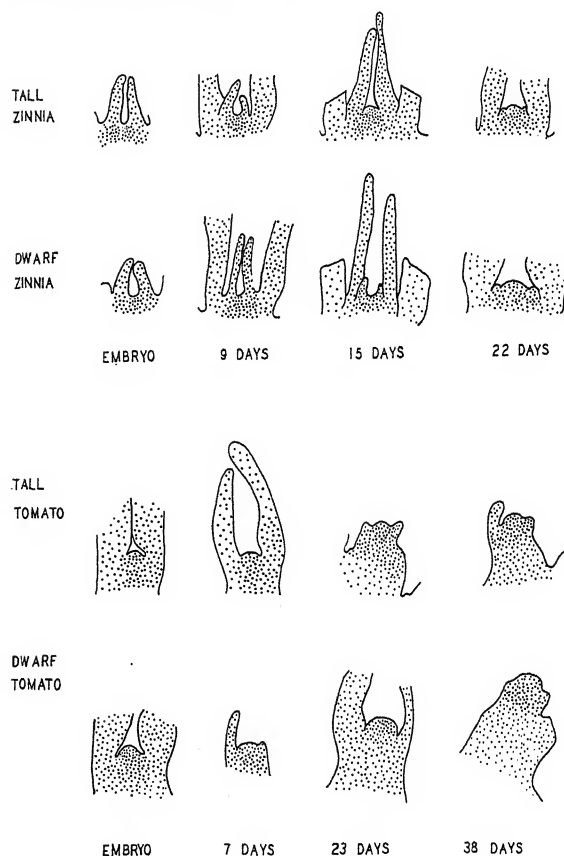


Fig. 7. Diagrams of growing points of Marglobe and Dwarf Stone tomatoes and Giant and Lilliput zinnias at four stages of development. $\times 60$.

MERISTEM SIZE IN TOMATOES AND ZINNIAS.—Observations of median longisections of typical meristems of zinnias and tomatoes at four stages of development (fig. 7) showed no striking differences in the meristem sizes of tall and dwarf forms. There seems to be, therefore, no correlation between meristem size and plant height. This is in agreement with the observations of Bindloss (1938) in which no relationship was found between meristem size and the respective vigor of inbred and hybrid maize plants. In the tomatoes there was a tendency for the meristems to be a little broader in the dwarf variety.

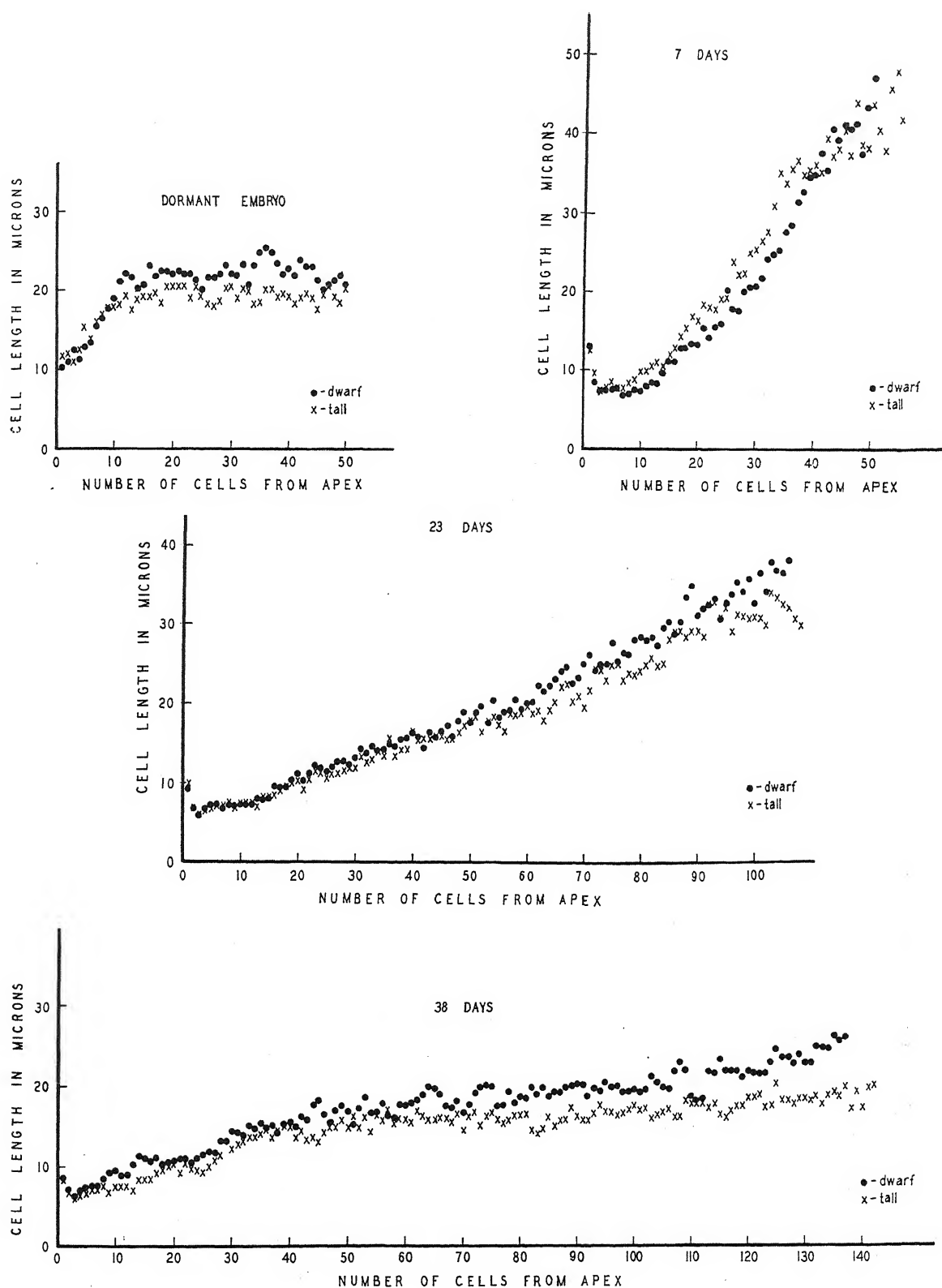


Fig. 8-11. Successive cell lengths in the terminal meristematic regions of Marglobe and Dwarf Stone tomatoes.—Fig. 8 (upper left). Dormant embryo.—Fig. 9 (upper right). Seven-day-old seedlings.—Fig. 10 (center). Twenty-three-day-old plants.—Fig. 11 (below). Thirty-eight-day-old plants.

These Dwarf Stone plants have stouter stems than those of Marglobe. This agrees with the observations of Houghtaling (1940), who found that the diameter of the primary body of a stem is determined at the stem apex where the new tissues are being formed.

CELL LENGTHS IN TOMATOES.—General trends.—The lengths of successive cells in the terminal meristematic regions of dwarf and tall tomatoes at four stages of development are shown in figures 8 to 11. It may be seen that the cells of the promeristem become shorter as the plant grows older. One might expect that as the cells at the apex decrease in length, those immediately behind this region would also be shorter than at preceding stages. This is the case for plants older than seven days, and as a plant grows older a much greater portion of the terminal part of the stem is occupied by cells which are shorter than comparable ones of younger plants.

A difference in the rate of division of comparable cells during the plant's ontogeny was suggested by the greater number of short cells in the apex of older plants as contrasted with the number in young plants. That is, where cell division proceeded most rapidly, the interval between divisions would be shorter and hence the cells would be smaller. Table 1

TABLE 1. Length of cell division zone in stem tips of Marglobe and Dwarf Stone tomatoes.

Age in days	Distance of last mitotic figure from apex (in microns)	
	Marglobe	Dwarf Stone
23	1,835	1,003
38	5,623	4,151

shows the extent of the region of cell division in stem tips of Marglobe and Dwarf Stone plants 23 and 38 days old. In both these varieties cell division extends over a region approximately 3,000 microns longer in plants 38 days old than in those 23 days old. Thus not only are the cells shorter in the apex of older plants, but, as a result of a longer zone of cell division, more cells are dividing.

Dormant embryos.—The first ten cells in the shoot apex of the two varieties were very uniform in length. However, cells 10 to 50 of Dwarf Stone plants, which lay in the unelongated hypocotyl of the embryo, were longer than comparable ones of Marglobe. The difference of the means of the two varieties was twelve times the standard error, the greater length of the Dwarf Stone cells being clearly significant and showing an inverse relationship between cell length and stem length.

Seven-day-old seedlings.—The promeristem cells of the two varieties were again equal in length. The cells behind the promeristem had elongated and formed part of the hypocotyl. However, Marglobe cells were now significantly longer than those of Dwarf Stone, a reversal from the condition observed in the dormant embryos. To accomplish this, Mar-

globe cells must have elongated more rapidly than those of Dwarf Stone. Some cell division must occur at the apex, however, for cells 1 to 35 were shorter than similar cells in the embryo. For Marglobe cells in the seedling to be longer than those of Dwarf Stone, their rate of elongation must have been faster during the period of germination, since presumably there was little division in the seedling.

Twenty-three-day-old plants.—At this stage of development comparable cells of Marglobe and Dwarf Stone plants were essentially equal in length up to the twentieth cell from the apex. All cells were clearly shorter than comparable ones of the preceding stage. Cells of Dwarf Stone at greater distances from the stem apex were slightly longer than those of Marglobe, but since the difference in their mean lengths was only about two times the standard error, the significance of this difference may be questioned.

Thirty-eight-day-old plants.—As in all preceding stages, the cells of the promeristems were of equal length for both forms. On the other hand, the tendency for older cells of Dwarf Stone individuals to be longer than those of Marglobe was even more pronounced. The difference in mean cell length for cells 40 to 115 was definitely significant, for it was ten times its standard error.

The cell lengths of these 38-day-old plants bear a definite relationship to plant height. The greater length of these Dwarf Stone cells indicates that cell division is going on less rapidly in the embryonic region of Dwarf Stone stem tips than in those of Marglobe. Not only are cell divisions in the dwarf plants less frequent, but they are also less numerous. There is a striking difference in the extent of the mitotic region of tall and dwarf plants which could be observed in this and in the preceding stage. In the seedlings mitotic figures were not readily observed, probably because growth of the hypocotyl results almost entirely from the elongation of cells which lie in the embryo, with only a limited amount of cell division at the apex. However, in the later stages, mitotic figures could be observed, and the approximate extent of the region of cell division could be determined (table 1). The mitotic zone of Dwarf Stone plants was always shorter than that of Marglobe, i.e., cell division is confined to a smaller portion of the stems. In plants 23 days old, mitoses were observed in a region more than 1,800 microns long in Marglobe plants, but only slightly above 1,000 microns in Dwarf Stone plants. In plants 38 days old, cell division figures were found in a region 5,500 to 5,600 microns long in Marglobe plants and 4,000 to 4,200 microns long in the dwarfs.

It is readily seen that at least in plants 23 and 38 days old there is a difference in the cell lengths in tall and dwarf forms which is a reflection of their respective rates of cell division. Furthermore, there is good evidence that the greater height of Marglobe plants is accounted for in large part by a longer region of embryonic cells in the stem tip, which, in contrast to the embryonic region of Dwarf Stone plants, gives rise to a greater number of cell generations. The difference then between tall and dwarf plants is estab-

lished in the growing points, since mature cell size is the same in both varieties.

CELL LENGTHS IN ZINNIAS.—General trends.—There is a remarkable similarity in the developmental pattern of cell lengths of zinnias and tomatoes. Successive cell lengths of each stage are shown in figures 12 to 15. Absolute cell size was not identical. The older cells of zinnia are generally longer than those of tomato, but the same trends were apparent. As in tomato, cells of the seedlings were longer than at any other stage. There was a slight decrease in length of the cells in the promeristem at each successive stage of development, but the trend was not as definite as in tomato. As the plants developed, the older cells, i.e., those farthest from the apex, were generally shorter than comparable ones at preceding stages, but the extent to which they were shorter was not as great as in tomato.

Embryos, seedlings, and plants 15 and 22 days old.—As in tall and dwarf tomatoes, cells 10 to 50 of nine-day-old seedlings of the Giant variety were longer than those of Lilliput, but these longer cells evidently do not establish a difference in height between the two forms. Comparable cells of the two varieties in the embryo were equal in length, in this respect differing from those of the tomato where the dwarf plants had the longer cells.

In zinnia, as in tomato, older cells of dwarf plants were longer than similar ones of tall plants. Commencing with the 45th cell from the apex of 15-day-old plants the Lilliput cells were significantly longer than Giant. This difference in cell length between Giant and Lilliput varieties was even more significant in 22-day-old plants. Because of the lack of visible mitoses in this material it was impossible to determine the extent of the region of cell division.

Although zinnias are not related to tomatoes in most respects, the similarity in the changes in cell lengths of their tall and dwarf varieties was outstanding. In both, the cell lengths in the promeristems decreased as the plants matured. Likewise cells of dwarf plants in the later stages were longer than those of tall plants. Further investigation is necessary to establish where there is a relationship between the lengths of cell division in regions in Giant and Lilliput zinnias which corresponds to that observed in Marglobe and Dwarf Stone tomatoes.

DISCUSSION.—Two aspects of the problem of cell length and its relation to plant height must have consideration here: (1) the changes of a general nature which occur during the vegetative ontogeny of tall and dwarf plants alike, and (2) the relationship of cell length to the problem of height differences which are hereditary.

General trends.—Cell lengths in the promeristems of zinnias and tomatoes, as has been noted, are progressively shorter as a plant grows older. This is contrary to the general conception that the cells in this region are equal in size at all stages of the plant's development. Whaley (1939) made a study of cell volume in promeristems of a series of tomatoes of vary-

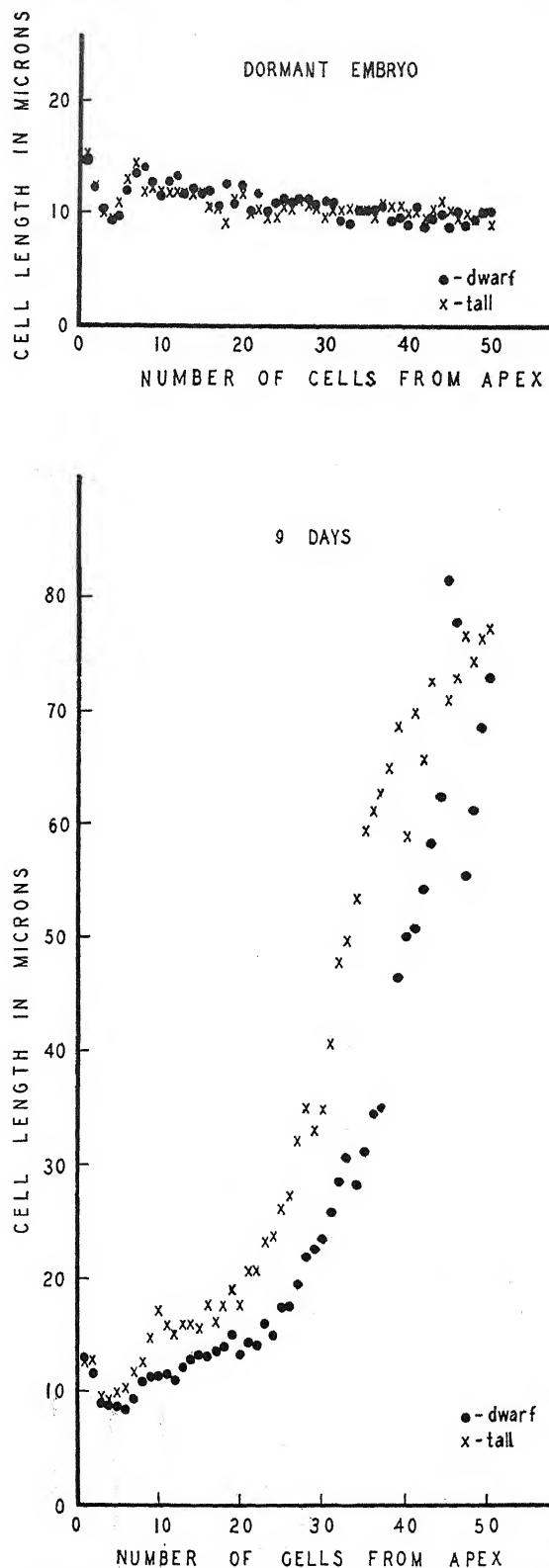


Fig. 12-13. Successive cell lengths in the terminal meristematic regions of Giant and Lilliput zinnias.—Fig. 12 (above). Dormant embryo.—Fig. 13 (below). Nine-day-old seedlings.

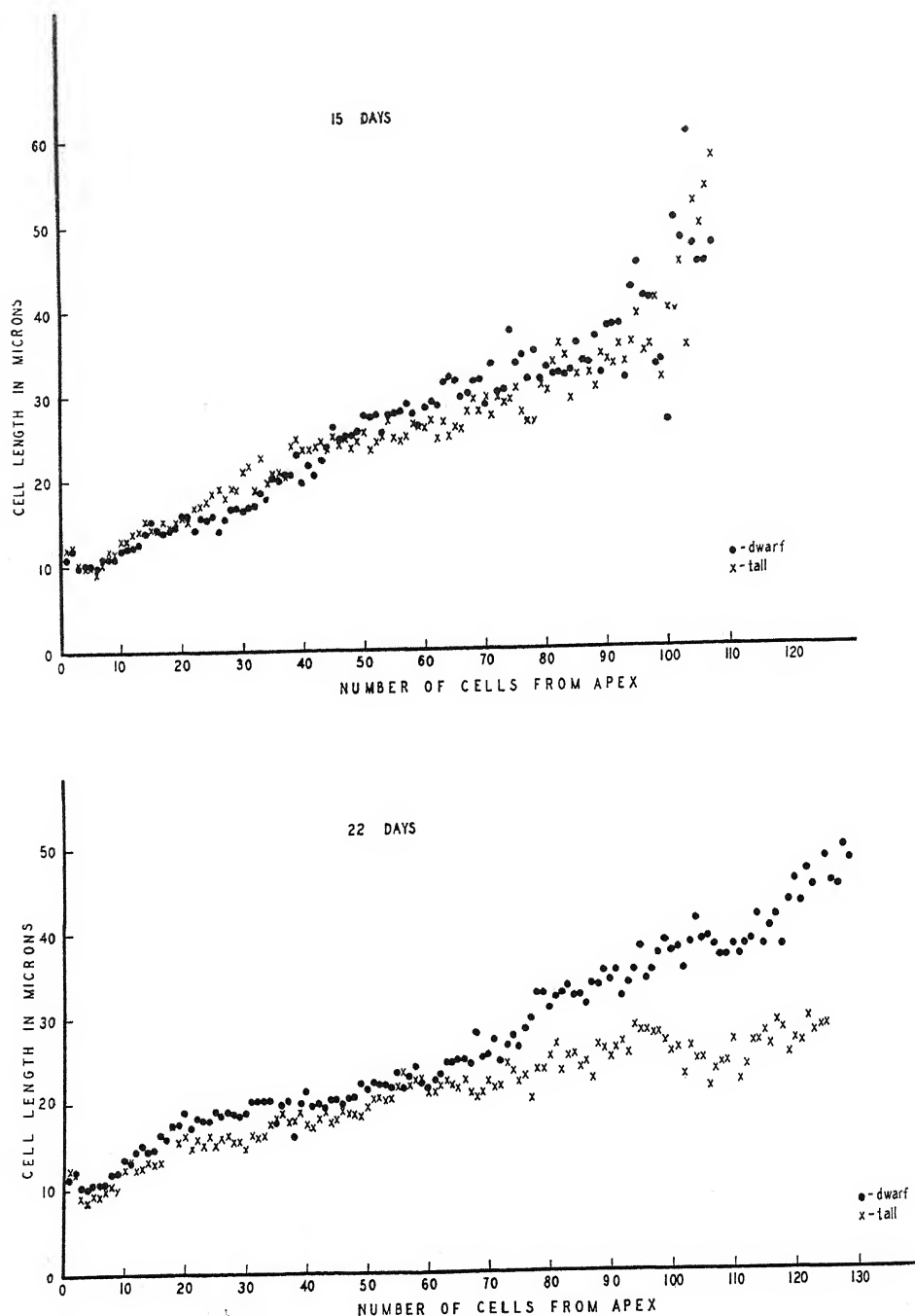


Fig. 14-15. Successive cell lengths in the terminal meristematic regions of Giant and Lilliput zinnias.—Fig. 14 (above). Fifteen-day-old plants.—Fig. 15 (below). Twenty-two-day-old plants.

ing ages and found that the volume of promeristem cells also decreased. He related this smaller volume of promeristem cells in older stem tips to a greater competition for food and food materials accompanying the increased size of the plant and the formation of more growth centers in which cell division was continually occurring.

The present investigation on cell size, in contrast to Whaley's, includes an analysis of cell lengths in the region of elongation and vacuolation as well as in the promeristem. As in the promeristem, cell lengths of both tomatoes and zinnias in this region also change as the plant matures, cells of one stage being regularly shorter than comparable cells of the

preceding stage. In these older plants the region of short cells extended farther down into the stem, and it was found that this was correlated with a longer embryonic region in the stem tips of older plants.

It is important to know that cell size in comparable regions is not the same for plants of different ages and phases of development. This fact has been overlooked prior to the relatively recent investigations in developmental analysis of cell and organ size relationships. Obviously where organ sizes are different, a study of comparative cell sizes should be based on materials of known age or state of maturation so that corrections may be made for normal changes in cell size. The problem of absolute cell size and its relation to organ size may not be as important as the relative size of cell to organ, and this can only be determined, as in the present study, by following cellular ontogeny. Furthermore, the lack of definition and consistency in the use of the term "cell size" has led to the present confusion concerning the relationship of cell size to organ size.

It is no longer possible to think that the chief center of cell division is in the relatively short zone 60 to 100 microns from the stem tip. Cells behind this region also divide although they are in various stages of vacuolation. Observations in the tomato stem tips showed many instances in which vacuolate cells 45 microns long were dividing. In fact, more mitotic figures were observed in vacuolate cells than in the regions of non-vacuolation at the apex. Lateral organs, such as leaves and axillary buds, are undoubtedly formed by cell division in the promeristem, but the activity of cells in this region is not solely responsible for stem length. Priestley (1929) was among the first to point out that vacuolate cells as well as non-vacuolate ones are capable of division in the stem apex.

Although the tomato and zinnia plants were quite different in size and appearance, the trends in the development of cell length in the stem apex are remarkably similar. Thus, although absolute cell size in these two genera is not identical, the behavior of the cells follows a very similar developmental pattern which operates throughout the whole period of growth.

Cell length and plant height.—There seems to be no consistent positive relationship between cell length and stem length. The cells of the promeristem are approximately equal in length in tall and dwarf forms. Comparable cells in the regions of elongation in tall and dwarf plants, however, show a definite sequence of shifting size relationships during development. It has already been seen that in the embryos of tall plants the cells were generally shorter than those of dwarf plants; that in the seedlings the situation was reversed, with cells of tall plants the longer; and that another shift occurred in the third developmental stage where cells of the dwarfs tended again to be longer. The general growth pattern appeared fairly well established and stable at that stage, for in the last stage prior to flowering, the greater length of cells in the dwarfs was even more pronounced. Thus,

it is obvious that the growth of cells in the shoot apex is a complicated process which is subject to modifications at various stages in the plant's ontogeny.

It has been shown that cell division is confined to a shorter region of the stem tip in Dwarf Stone individuals. This seems to account for the longer cells of dwarf plants 23 and 38 days old. Where cell division stops early and is followed by elongation, the cells will be longer than in individuals where cell division extends over a greater region and maturation and elongation are not allowed to go to completion. Thus, tallness in the Marglobe plants is probably due to a greater number of cell generations, since observations on mature cell size showed no differences in the cell lengths of tall and dwarf varieties.

Observations on the extent of cell division do not rule out the possibility that there may be different rates of cell elongation in Marglobe and Dwarf Stone varieties. For example, Anderson and Abbe (1933) have shown a definite case in which size of cell was determined by the rate of secondary wall formation which in turn limited cell elongation. No evidence was obtained on secondary wall formation or actual rate of elongation in the tomato varieties here studied.

Certain physiological processes such as rate of auxin production may differ in tall and dwarf plants. Since there is evidence that auxin is related to cell elongation and, therefore, presumably to plant growth, it may play an important rôle in determining tallness and dwarfness. Indeed, van Overbeek (1935, 1938) studied dwarfness in maize from a hormonal point of view and found that normal and *nana* forms produced equal amounts of auxin in the early stages, but that the dwarf variety *nana* destroyed it more rapidly.

In the present study, the use of the *Avena* method and diffusion technique in preliminary investigations on the hormone content of a number of tall and dwarf forms showed some indication that there was more growth substance in the tall varieties. This research has not been carried on because the diffusion method leaves much to be desired in quantitative determinations of growth hormone content. Research on the hormonal aspect of plant height awaits the development of suitable extraction techniques for particular green tissues.

In the zinnias the differences in height between Giant and Lilliput forms were not pronounced in the early stages. However, elongating cells were comparable in their behavior to those of tomatoes. Whatever may be the explanation for this lack of height difference (see paragraph 6, page 181), the significant fact seems to be the similarity in the developmental pattern and cellular organization at the shoot apex of "tall" and "dwarf" zinnias. One might expect, because there are no height differences between the zinnias used as material in this study, that their internal pattern of development would be identical. However, such is not the case, for by analogy with the tomato varieties, the internal development of the

embryonic tips of Giant and Lilliput plants is the same as though they had actually developed into tall and dwarf individuals. Thus there is a fundamental difference in the embryonic tissues of Giant and Lilliput stem tips, but this difference was not translated into actual height differences under the existing environmental conditions.

Sinnott (1940) has observed that in small and large fruited cucurbits there is also a fundamental developmental pattern which proceeds regularly regardless of the manner in which the substance of the organism is divided up. In the zinnias studied the factors responsible for inhibiting their potential development must be those which act on the differentiation and maturation processes of cellular ontogeny, since in their early cellular ontogeny both tall and dwarf plants are similar.

In viewing the organism as a whole one may conclude from this investigation that there is a basic histological pattern of development, fundamental in organization, and modified in various ways in response to genetic changes. While the problem of the relationship of mature cell size to mature organ size is an important one in the study of size differences, it merely represents the end product of a whole series of changes. Only a greater knowledge of the processes of development and differentiation and the changes which occur in the ontogeny of both cells and organs will make it possible to interpret the organism as a whole in terms of its parts.

SUMMARY

A developmental study was made of cell lengths in the apical meristematic regions of genetically tall and dwarf races of *Lycopersicon esculentum* and *Zinnia elegans* during their early growth and development. The tall and dwarf plants in each case were grown from seeds of essentially equal weight. Measurements of series of successive cells were made (in embryos, seedlings, and older plants), extending from the apex in a proximal direction, to determine

whether there was a relationship between cell length in meristematic regions and mature stem length.

In the embryo and at all later stages of development, Marglobe tomatoes were always taller than Dwarf Stone. The Giant zinnias were not appreciably taller than Lilliput until flower formation.

No difference in meristem size was apparent between tall and dwarf plants.

Cell lengths of the first ten cells in the series, forming the promeristem, decreased as the plants grew older. These cells were equally long for tall and dwarf varieties, thus showing no correlation with plant height.

Cells were elongating directly behind the promeristem of both zinnias and tomatoes, but their lengths at successive stages in the plant's ontogeny were less as the plant matured. These shorter, vacuolate cells in the older stages of Marglobe tomato plants were associated with a longer meristematic region, thus accounting for the greater height of these plants by making a greater number of cell generations possible. In contrast, embryonic cells of Dwarf Stone are longer because they attained maturity sooner, and thus stopped dividing.

There was a series of shifting size relationships of the vacuolate cells in tall and dwarf plants, indicating that tallness and dwarfness are not due to absolute differences in lengths of cells at the apex.

Although there were no sharp differences in total height of Giant and Lilliput zinnias, the behavior of the cells in their respective meristems was essentially the same as that of tall and dwarf tomatoes. Thus, while there is no constant relationship of cell length to stem length during vegetative ontogeny, there is, nevertheless, a fundamental and genetically controlled sequence of changing size relations in the terminal meristems which is ultimately related to differences in cell number and thus to differences in plant height.

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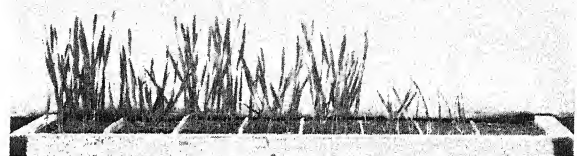
HEREDITARY SUSCEPTIBILITY TO X-RAY INJURY IN TRITICUM MONOCOCCUM¹

Luther Smith

EXTENT AND NATURE OF INJURY.—In the fall of 1939 dormant seeds of three stocks of einkorn were X-rayed simultaneously for a study of mutation rate. One was much more severely injured by the treatment (7,500 r-units) than the other two. The greater susceptibility of this stock to X-ray injury has since been confirmed in comparisons with various other stocks and has been shown to be conditioned by a gene. This factor has been called "X-ray susceptible" or "susceptible" (symbol *xs*). Following treatment of dormant seeds the injury takes the form of more pronounced flecking on the first leaves, slower growth, and death from smaller doses than are required to kill normal seeds.

In one test, treatments of 4,000 r, 8,000 r, and 12,000 r-units were applied to dormant seeds (thirty seeds per group) of the susceptible and of a non-susceptible stock. After ten days, seedlings from the susceptible line rayed 8,000 r were about half as tall as those given 4,000 r. None grew from the seeds given 12,000 r. In the resistant line there was only a slight difference between the 4,000 r and 8,000 r groups, while the 12,000 r group was about half as tall as the 4,000 r group. In another trial an early-maturing line and the susceptible stock were compared. Treatments of 5,000 r, 10,000 r, and 20,000 r were applied. Sixty seeds were given each treatment and divided into two series, thirty seeds per treatment. After they were rayed, the seeds were soaked in tap water at about 22°C. for twenty-four hours and then planted in soil flats together with untreated checks. From figure 1 it is apparent that the early-maturing stock grew somewhat more rapidly than the susceptible (legend for fig. 1). This was due in part to better seed, but some of the difference was due to the earliness characteristic. Neither stock was distinctly injured by the 5,000 r treatment, and the early was reduced in height only slightly by 10,000 r while the susceptible line was severely damaged. The

normal (early) line was reduced in height by 20,000 r to about the same degree that the susceptible line was by 10,000 r, while only a few coleoptiles emerged from the susceptible line given 20,000 r. The two series were practically identical. The data on emergence and height of plants are presented in table 1.



A A' B B' C C' D D'

Fig. 1. Ten-day-old seedlings of an early (normal) stock and an "X-ray susceptible" stock of einkorn. A (normal), A' (susceptible), no treatment; B, B', 5,000 r; C, C', 10,000 r; D, D', 20,000 r. Note the serious injury to the "susceptible" line given 10,000 r though the normal line given the same dose shows little, if any, injury. See text.

The two series were combined in obtaining the values. Thus each percentage represents sixty treated seeds. A number of other trials were made, and each demonstrated clearly the greater injury to the susceptible line.

INHERITANCE OF SUSCEPTIBILITY.—The susceptibility has been demonstrated in three generations. It apparently is recessive, as 138 seeds of the constitution *xs xs* × ++; 118 ++ × *xs xs*; and 116 ++ × ++ produced seedlings which were indistinguishable following treatments of 8,000 r or 10,000 r, though they were clearly less injured than the homozygous susceptible given the same treatments.

Further evidence that susceptibility is recessive and evidence that only one gene is concerned was obtained by X-raying seed from F₁ plants. The ratio of relatively uninjured to severely injured seedlings was approximately 3:1.

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TABLE 1. Comparison of the effects of X-rays on emergence and height of seedlings from a normal and an X-ray susceptible line of einkorn.

Treatment	Seeds producing seedlings		Average height in terms of the untreated check	
	Normal	Susceptible	Normal	Susceptible
	%	%	%	%
Check	100	100	100	100
5,000 r	104	94	103	92
10,000 r	100	84	94	42
20,000 r	88	16	32	4

TABLE 2. *A comparison of the induced mutation rate of a normal and the "susceptible" stock. (Not adjusted for size of head progeny.)*

Stock	Treatment	Head progenies	Seedlings per progeny	Mutants	Heads with mutants
		No.	No.	No.	%
Normal	5,000 r	523	16.6	17	3.3
Susceptible	5,000 r	466	11.6	13	2.8

Additional evidence on both of these points was obtained from treating seed from twenty-four F_2 plants (fifty seeds from each plant). As would be expected, the classification of the F_2 plants into homozygous and heterozygous susceptible and non-susceptible was in some instances uncertain, but the differences among their progenies were distinct, and the classification fitted reasonably well the postulated 1 homozygous non-susceptible : 2 heterozygous susceptible : 1 homozygous susceptible. Five plants were classified as $Xs Xs$, thirteen as $Xs xs$, and six as $xs xs$. These populations also supplemented the F_2 data in indicating that some plants gave progeny in the ratio of 3 normal : 1 susceptible.

TESTS FOR LINKAGE OF SUSCEPTIBILITY FACTOR.—The factor for susceptibility was first found in a "glume-awned" (Smith, 1939) mutant stock. However, some glume-awned plants seem not to be "susceptible"; hence, the two characters are not two effects of the same gene. Also in F_2 and F_3 populations seedlings which were homozygous for a recessive mutant gene, js , very closely linked in repulsion with the glume-awned factor (ga) which came into the cross with xs , were "susceptible." In another population segregating xs and $gl-2$ (Smith, 1939), known to be unlinked with ga , the $gl-2 gl-2$ seedlings were susceptible. Thus xs appears not to be linked with either of these unlinked genes, and further tests are necessary to find a linkage for it.

EFFECT ON MUTATION RATE.—As xs has an effect on the susceptibility of seeds to X-ray injury, and as it is known that X-rays have distinctly different effects on the mutation rates of organisms (Stadler, 1931) and that genes may alter mutation rates (Demerec, 1937), it was thought possible that the susceptibility to injury might be associated with an in-

creased mutation rate. However, in preliminary tests there was no appreciable effect of the gene on mutation frequency.

Dormant seeds of the susceptible and a normal stock simultaneously were given a treatment of 5,000 r units and planted immediately. Head progenies of the resulting plants were grown to the seedling stage. About 2.8 per cent of the head progenies of the susceptible line were segregating for a conspicuous mutant (white, yellow, virescent, etc.) while 3.3 per cent of the head progenies of the normal line contained mutants (table 2). However, the normal line averaged 16.6 seedlings per head progeny as compared to 11.6 for heads of the susceptible line. Additional data from segregating susceptible and other normal stocks also indicated that there were probably no significant differences in mutation rates (table 3), though two of five segregating stocks (e and h) had a higher frequency of mutations.

X-RAY SUSCEPTIBILITY FACTOR AND HEAT TOLERANCE.—Some investigators (e.g., Shkvarnikov and Navashin, 1935) have likened X-rays to heat in the production of effects in living organisms. Therefore, the effect of heat on the X-ray susceptible and other stocks was compared. The "susceptible" stock was not especially susceptible to heat injury. Rather the reverse was true. In one series of treatments comparing the effect of high temperatures on the viability of dormant seeds of six stocks of einkorn (fifty seeds per treatment), the "susceptible" line was equal in viability to the best of the stocks at 50°C. and 60°C. for five and ten days. It was the only stock which germinated at all after five days at 70°C. In this comparison the seeds were germinated immediately after removal from the ovens. In another series in which the seeds were allowed a day between treat-

TABLE 3. *A comparison of the induced mutation rate of several normal and several segregating "susceptible" stocks. (Not adjusted for differences in size of head progeny or dose.)*

Stock	Treatment	Head progenies	Seedlings per progeny	Mutants	Heads with mutants
		No.	No.	No.	%
++ a	5,000 r	119	12.9	3	2.5
++ a	7,500 r	117	9.3	3	2.6
++ b	7,500 r	433	23.4	23	5.3
++ c	7,500 r	431	11.9	11	2.6
+ /xs d	5,000 r	53	20.3	2	3.8
+ /xs e	5,000 r	204	16.0	10	4.9
+ /xs f	5,000 r	85	16.4	0	0.0
+ /xs g	5,000 r	333	14.0	10	3.0
+ /xs h	5,000 r	151	12.6	8	5.3

ment and germination, the "susceptible" line was not outstanding in viability.

DISCUSSION.—As Sax and Swanson (1941) point out, sensitivity to X-rays has been of two sorts: (1) The physiological or killing effect and (2) the genetic effect. The factor here reported in einkorn apparently affects only the physiological sensitivity. Hence, the discussion will be limited to a small part of the literature dealing with physiological sensitivity and the factors which modify it. Certainly some of the factors could not be operating in the material reported here but are mentioned in order to indicate the types of things which affect sensitivity.

Physiological sensitivity to X-rays varies greatly among species and genera and even within species. Johnson (1936a, b) studied the sensitivity of 100 species distributed among fifty families. Of seventy species which were compared directly, fifteen were unaffected, fifteen were slightly affected, and forty were noticeably affected by X-rays under the conditions imposed. She also found (1933) differences in the sensitivity of three varieties of *Atriplex hortensis*. A red and white were more severely injured than a green variety. Mavor (1927) reported that female pupae of *Drosophila melanogaster* were more resistant to X-rays than male. Stadler (1929) observed differences in survival of seeds of several species of wheat and oats following X-ray treatments. The polyploid species were more resistant to injury than the diploid.

It is well established that the conditions of treatment or state of the individual or tissue has an effect on the tolerance for X-rays. Stadler (1931) reported that dormant barley seeds tolerated a dose fifteen to twenty times as great as germinating seeds. Soaked seeds were more sensitive than dry, dormant seeds.

Johnson (1926) reported that catalase activity was decreased by x-irradiation while oxidase activity was unaffected in *Helianthus annuus*. She (1933) determined that the greater susceptibility of a red and

a white variety of *Atriplex hortensis* as compared to the green (above) was paralleled by a greater decrease of catalase activity in the former. Cattell (1931) reported that vitamin B in wheat embryos was destroyed by heavy doses of X-rays. Skoog (1935) observed that X-irradiation inactivated auxin.

The mechanism by which the factor herein reported increases the susceptibility to X-ray injury (though apparently not affecting mutation rate or susceptibility to heat injury) has not been determined. It is not due to higher water content. Seeds of the early (normal) line and the susceptible line (fig. 1) had 11.7 per cent and 11.5 per cent moisture respectively. Also the meiotic chromosomes of the susceptible line appeared to be the same size as those of normal stocks. It is hoped the susceptibility can be analyzed as a controllable alteration in the chemical constitution or hormone or vitamin relations.

The factor here discovered may have some bearing on the difficulty encountered by investigators in duplicating the results of others involving studies of X-ray tolerance. These difficulties heretofore have frequently been attributed to differences in apparatus rather than to differences in the genetic or physiological constitution of the organism.

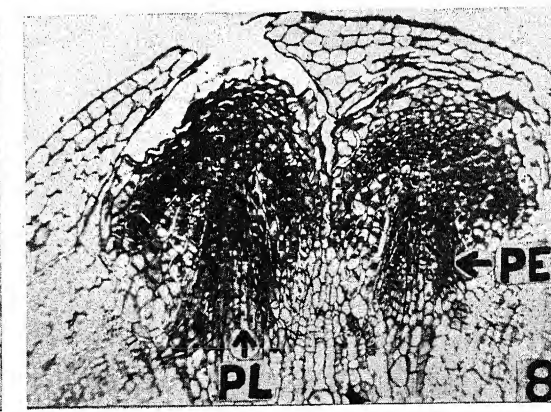
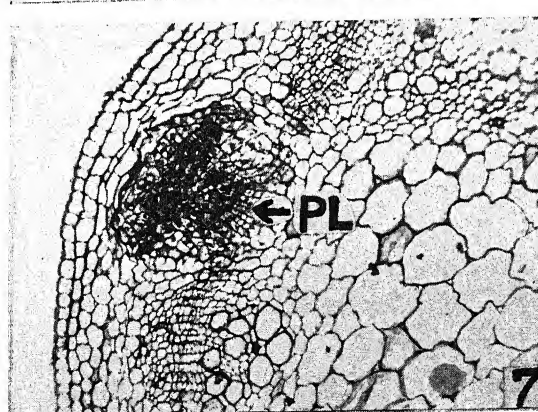
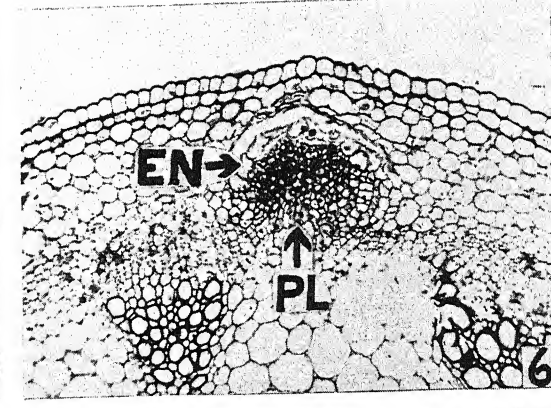
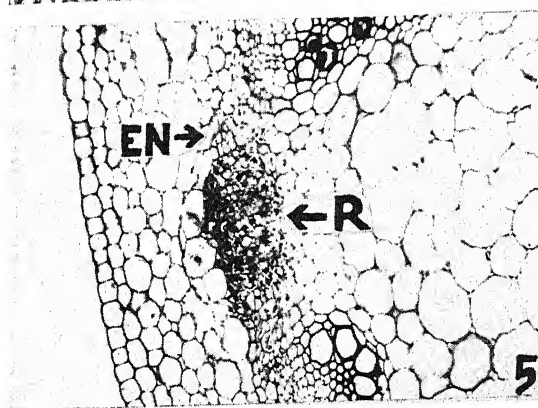
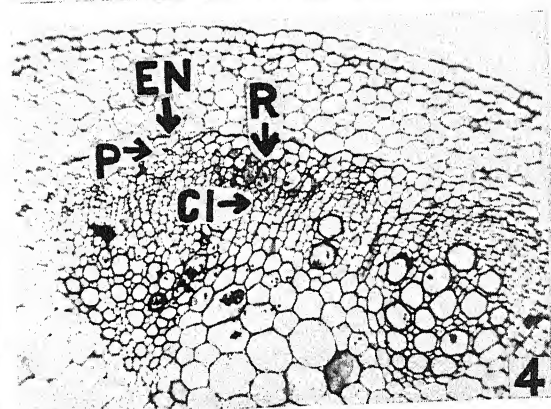
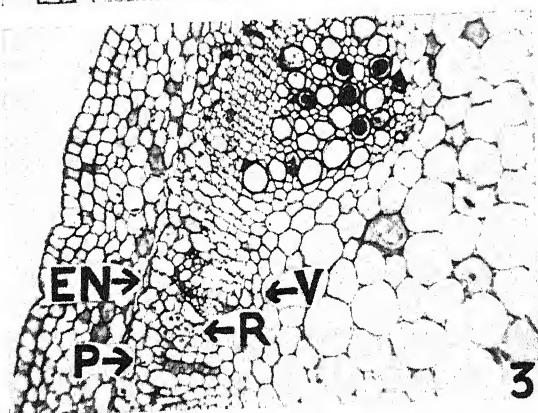
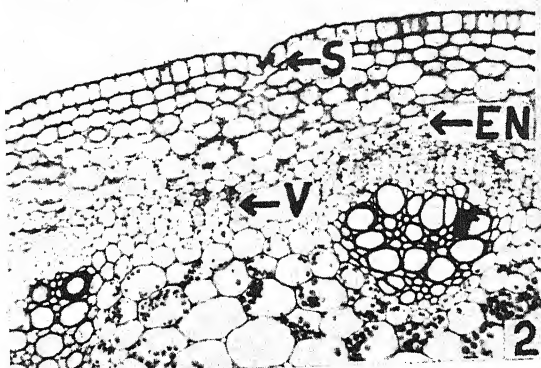
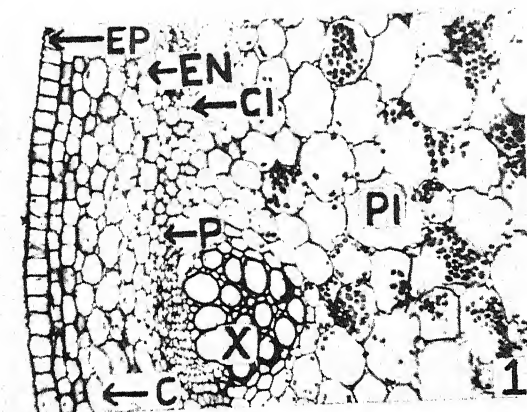
SUMMARY

A mendelian factor was found in einkorn wheat (*Triticum monococcum*) which distinctly increased the susceptibility of dormant seeds to X-ray injury. In preliminary tests the factor had no apparent effect on mutation rate nor on susceptibility to heat injury.

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IT HAS been determined that adventitious roots in stem cuttings may arise in many different tissues of the stem, nearly always in association with cells near the conducting tissues. This condition also maintains in *Tropaeolum majus* L. This paper has been prepared because it was noted that adventitious roots arise from stem cuttings of the garden nasturtium in a somewhat shorter time than is required in many other herbaceous plants. With no chemical treatment roots emerge from stem cuttings in five to seven days.

Since little has been added to the literature during the past year, attention is called to Swingle's (1940) rather exhaustive review concerning various phases of regeneration and vegetative propagation.

MATERIALS AND METHODS.—Cuttings were made from stems of various ages and placed in slightly alkaline tap water. In about five days roots began to emerge from the basal 2 to 3 mm. of the stem cutting. Somewhat later, roots appeared farther up the stem, mostly at the nodes. In most cases the roots emerged at right angles to the stem axis. Material was taken from the basal 2 mm. of the cuttings, fixed in formalin-acetic acid-alcohol and imbedded in paraffin. Sections were made at thicknesses varying from 5 to 20 microns, those at 15 microns proving most satisfactory. Staining was done with safranin and fast green. Photomicrographs were taken with a Leica copying attachment upon Eastman Bibliophile film. No filters were used.

The Salkowski indol test, as commonly used by bacteriologists, was used on freshly harvested *Tropaeolum* plants without any indication that indol is produced naturally. It was thought that such production may account for the rapid root formation.

STEM ANATOMY.—In *Tropaeolum majus* L. the single-layered epidermis has sparsely scattered stomata (fig. 2) and rather small substomatal chambers. The cortex consists of six to eight layers of cells between a distinct endodermis and the epidermis (fig. 1). Probably due to sectioning, the endodermal cells were found to have collapsed in a few cases (fig. 3, 4). Although some starch grains are present in the

cortex, they are not as abundant here as in the outer areas of the pith (fig. 1, 2). These starch grains disappear after the cuttings have remained in the water for two or three days (fig. 3, 4). The pericycle is a rather poorly defined tissue (fig. 1, 3) adjacent to the definite endodermis with Casparian strips (fig. 1, 2).

Vascular bundles are separated by medullary rays in young stems, but the interfascicular cambium of the older stems makes complete cylinders of conducting tissue. Small vascular bundles are frequently found between the larger ones (fig. 2, 3).

The pith of the stem is quite large and, as mentioned above, is abundantly supplied with starch grains (fig. 1, 2).

ADVENTITIOUS ROOTS.—Adventitious roots make their appearance comparatively early, although there is no evidence of preformed root primordia. Cuttings that had been in water three to four days were best suited to the study of root initials (fig. 3, 4).

Roots are always found to appear in the interfascicular region between the endodermis and the interfascicular cambium when the latter is present (fig. 2, 3). At first it is difficult to distinguish between a very small vascular bundle and a root primordium (fig. 3). In the root primordium the nuclei are larger, the protoplasm more dense, and the cells larger than in the phloem of the vascular bundle. Both have a similar location in the stem cuttings (fig. 3).

The youngest root primordia are distinguishable before any meristematic activity of the pericycle is noted. These primordia are located in the ray parenchyma cells between the pericycle and the interfascicular cambium when the latter is present (fig. 3, 4). Since these parenchyma cells normally give rise to interfascicular cambium, they possess meristematic potentialities.

As meristematic activity continues, the cells between the root primordium and the endodermis enlarge and also become meristematic. These cells which undoubtedly are pericyclic become part of the root and lose their identity as pericycle (fig. 5). Cells of the endodermis enlarge greatly as the root increases in size (fig. 5, 6), but these cells do not undergo mitoses. Shortly after the endodermal cells enlarge, a pocket appears in the cortex ahead of the young root (fig. 6). The endodermis thereafter soon

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Fig. 1-8. All figures are of cross sections of the stem, the young roots being sectioned longitudinally. All magnifications are $\times 565$. Epidermis, EP; cortex, C; endodermis, EN; pericycle, P; interfascicular cambium, CI; xylem, X; pith, PI; young vascular bundle, V; root primordium, R; plerome, PL; periblem, PE; stomate, S. Fig. 3-6 are from stems 4 days after cuttings were made and fig. 7, 8 at 6 days after cuttings were made.—Fig. 1. Cross section of normal stem.—Fig. 2. Normal stem showing stomate (S) and young vascular bundle (V).—Fig. 3. Root primordium (R) beside a young vascular bundle (V). Note pericycle (P) outside of root primordium.—Fig. 4. Root primordium similar to figure 3.—Fig. 5. Young root from four-day cutting showing enlarged endodermis (EN) of the stem. Compare with figure 6.—Fig. 6. Similar to figure 5 but plerome (PL) becoming more definite. Pocket has appeared outside of endodermis (EN).—Fig. 7. Similar to figure 6; endodermis and several cortical cells have collapsed.—Fig. 8. Histogens have definitely appeared in both roots that arose side-by-side.

disintegrates. No feasible chemical test was devised to ascertain whether the endodermis secretes a cellulose digesting enzyme which may aid the root in its emergence. Similar conditions were observed in *Begonia* cuttings (Smith, 1936), but Priestley and Swingle (1929) and later Swingle (1940) seem to doubt the possibility of adventitious roots being able to emerge by any fashion other than mechanical pressure. In *Tropaeolum* and *Begonia* it seems that both mechanical pressure and digestion work together in root emergence, thus accounting for such pocket formation in the earlier stages.

Root histogens appear before the root has emerged from the cortex, the plerome being the first to appear (fig. 6, 7, 8). The periblem is distinguished, and the root cap is in evidence before emergence (fig. 8). Nuclear divisions of the pericycle at the periphery of the primordium indicate that this tissue may have something to do with periblem and root cap formation while the initial cells give rise to the plerome.

SUMMARY

Adventitious roots in stem cuttings of *Tropaeolum majus* L. originate in the ray parenchyma between a

poorly defined pericyclic region and the interfascicular cambium when such is present. The pericycle later adds to the root initial. The endodermis ahead of the root primordium enlarges but does not undergo mitoses. A pocket appears ahead of the young root, outside the endodermis, indicating that cellulose digestion probably takes place, thus aiding root emergence. Histogen differentiation occurs before the root emerges from the stem.

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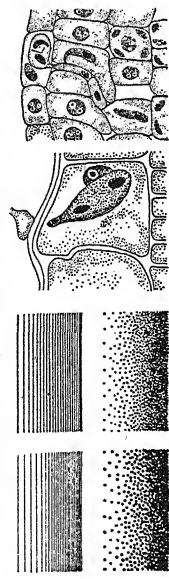


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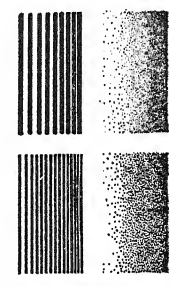
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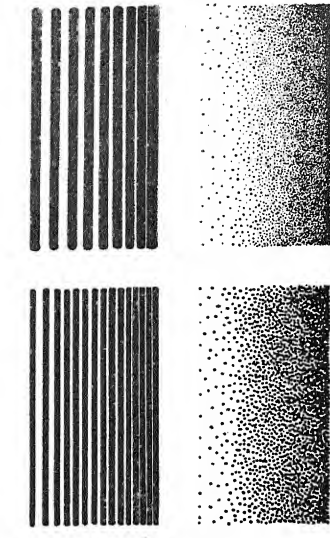
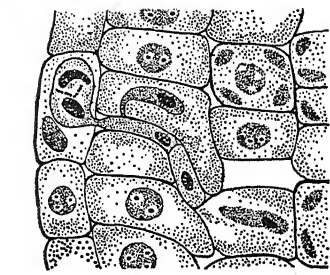
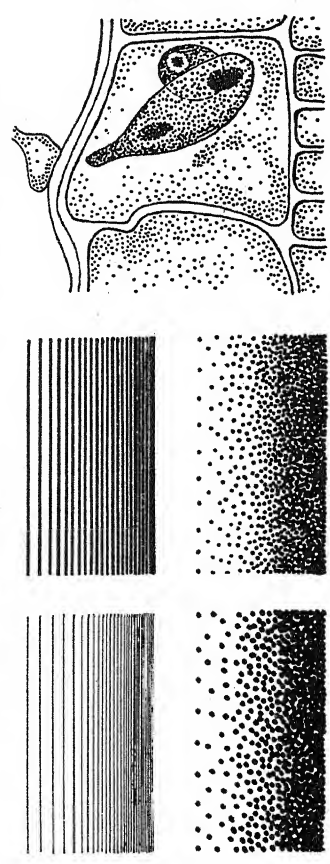


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DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

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Note that thin black lines hold up fairly well in reduction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. Keep the shading rather open. The degree of reduction needs to be known before the drawing is inked in.

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A DEVELOPMENTAL ANALYSIS OF INHERITED LEAF PATTERNS IN *TROPAEOLUM*¹

W. G. Whaley and C. Y. Whaley

MUCH ATTENTION has been directed of late to study of the mechanisms by which genes control the development of specific patterns. Leaves of certain plants, in which growth is essentially two-dimensional, present admirable material for such studies. Hammond (1941) has recently made an extensive investigation of the genic control of leaf shape in *Gossypium*. Developmental changes in the form of the leaves of *Tropaeolum majus* L. have been studied in considerable detail by Smirnov and Zhelochovtsev (1931) and by von Papen (1935). The genetic background of certain of the many leaf shapes of the genus *Tropaeolum* has been determined by the writ-

but are characterized by five lobes as seen in figure 3. As has been reported previously (Whaley, 1939) the hybrid from a cross between these two species bears leaves which are the same shape as those of *T. majus* but are larger than those of either parent. The F₂ from this cross was found to contain, in addition to plants with leaves similar in shape to those of each parent, plants bearing a third type of leaf. This new leaf is considerably smaller at maturity than either of the other types and is sharply or acutely lobed (fig. 2). Genetical analysis of this and other crosses has indicated that leaf shape here is determined mainly by two genes in epistatic rela-

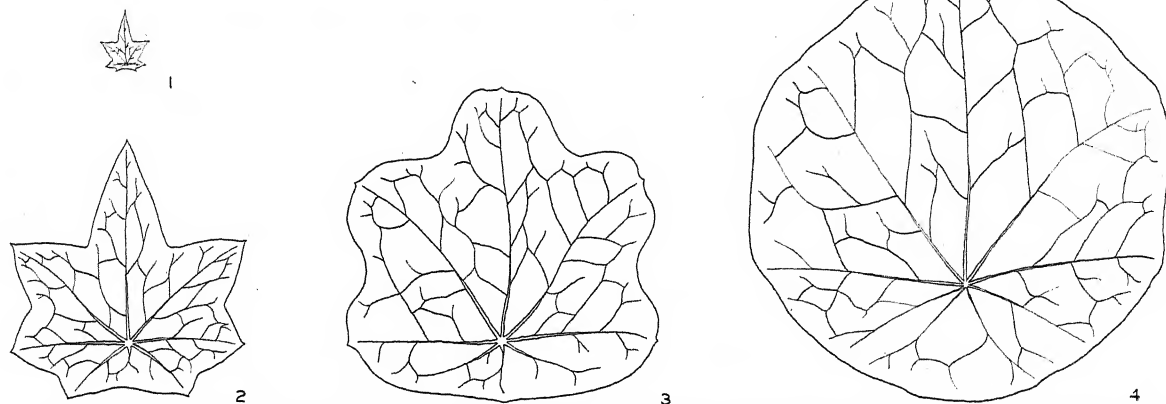


Fig. 1-4.—Fig. 1. Juvenile leaf.—Fig. 2. Acutely lobed leaf, *lu*.—Fig. 3. Roundly lobed leaf, *lU*.—Fig. 4. Orbicular leaf, *LU* or *Lu*.

ers and was reported in an earlier paper (Whaley, 1939). The data reported here are the result of an attempt to study intensively the ontogenetic form changes of leaf types whose genetic background is known, and to correlate the findings in such a manner as to obtain some idea of how the genes concerned operate in the development of shape differences.

MATERIALS AND METHODS.—*Tropaeolum majus* L. var. Golden Gleam bears leaves which are broadly orbicular in outline and which are relatively large when compared with the leaves of other species of *Tropaeolum* (fig. 4).

T. peltophorum var. *fimbriatum*² has leaves which are of the same basic shape as those of *T. majus*

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The authors wish to express their appreciation to the Massachusetts State College for financial assistance which made this study possible.

² *T. peltophorum* var. *fimbriatum* is also known as *T. Lobbianum fimbriatum*. Neither name has taxonomic validity. The former name is being maintained simply because the authors have used it in previous papers. The plant is of obscure hybrid origin, but it is homozygous for all the factors studied.

tionship. These genes have been designated as *L-l* and *U-u*. *L* produces orbicular leaves (fig. 4), and *U* in the absence of *L* produces roundly lobed leaves (fig. 3). Individuals which have the genetic constitution *lu* have acutely lobed leaves (fig. 2). Thus *LU* and *Lu* individuals have orbicular, *lU* roundly lobed, and *lu* acutely lobed leaves.

It was noted early in the course of the investigation that the juvenile leaves of all of these types are identical in appearance. Shape can be determined accurately when the leaves are between 1 mm. and 2 mm. in length, and at this stage all the types are similar (fig. 1). These juvenile leaves are very acutely lobed and are somewhat longer than wide.

Five plants of each of the three leaf types were selected for the experiment. On each of the fifteen plants selected the youngest unfolded leaf was measured in the following manner. The point of petiole attachment was taken as a base and the distance from it to the tip of the leaf was recorded as distance B, to the base of the next adjacent sinus, G, the tip of the next lobe, F, etc., as shown in figure 5. The lines

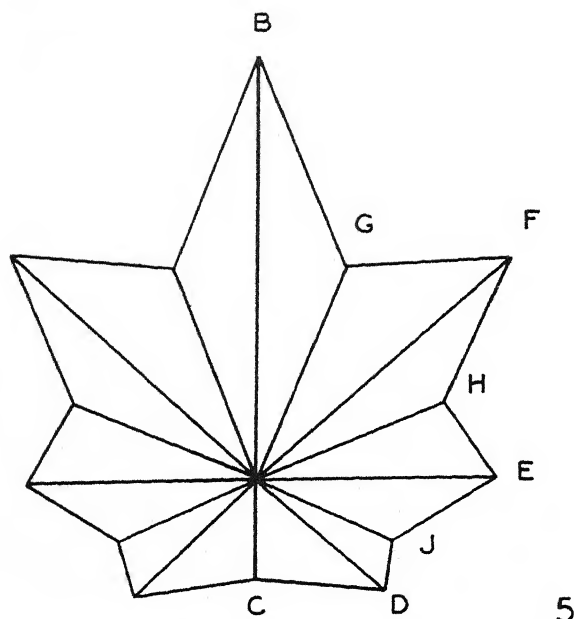


Fig. 5. Diagram showing measurements made on each leaf. See text for explanation.

B, F, E, and D correspond to the principal veins of the leaf, while lines G, H, J, and C indicate the points of greatest indentation of the sinuses, except where during the later stages of development these points disappeared. The measurements were then taken along a line approximately halfway between each successive pair of veins. A complete set of measurements was made daily on each leaf. As soon as the next youngest leaf on each plant had opened enough, measurements of it were begun, and in a similar manner five successive leaves on each plant were measured. Thus, daily measurements from the 1 to 2 mm. stage to maturity were made on twenty-five leaves of each type. The plants on which the measurements were made were grown under uniform field conditions and all measurements taken during the same period of the growth cycle. In the few instances where any evidence of injury to leaves by handling or insects appeared, the measurements were immediately discarded and the next uninjured leaf was measured.

HETEROGONIC GROWTH.—Using the growth along the midrib from the point of petiole attachment to the tip of the leaf as a common base, growth along each line was compared with it. The comparison was made by plotting upon logarithmic paper growth along line B on the abscissae against growth along each of the other lines in turn on the ordinates. In making the curves, the successive points for an individual leaf were first plotted out. Then measurements for the four other leaves on the plant were plotted on the same sheet. Finally the measurements from five leaves on each of four additional plants of the same genotype were superimposed on the same grid. Each graph line thus represents the average of twenty-five leaves. On the graphs the leaf types are

numbered as follows: 1—acutely lobed, 2—roundly lobed, 3—orbicular. The figures following the graph line numbers are k values (Huxley, 1932) indicating the ratio of the growth rates.

Thus in figure 6, line 1 represents growth along the line from the point of petiole attachment to the point of greatest indentation of the sinus, G, compared with growth along the main vein of the leaf from the petiole attachment to the tip of the leaf in an acutely lobed leaf. The k value of 1.01 indicates that growth along these two lines is proceeding at approximately the same rate. The slope of line 3 in this same figure ($k=1.30$) shows that in the orbicular leaves growth along G is much more rapid than growth along B. The figure indicates, then, that in leaf type 1, the acutely lobed leaf, growth in the terminal lobe is at about the same rate as growth in the adjacent sinus area, but that in the roundly lobed leaf growth, during the period measured, proceeded more rapidly in the sinus area than in the lobe ($k=1.14$), and in the orbicular leaf growth is still more rapid in the sinus than in the lobe ($k=1.30$). Figure 7 shows that in all three types of leaves the terminal lobe and the next adjacent lobe, F, grow at about the same rate. Figure 8 represents growth of the second sinus, H, plotted against growth of the terminal lobe and indicates that relatively this sinus grows at about the same rate as G. Figures 9, 10, 11, and 12 show the growth of the other lobes and sinuses plotted in this same manner.

Next, the angles between the veins of the various leaves were measured and found to be constant, at least in the proximal parts of the veins. Not only were the angles constant, but approximately the same angles were found in all the leaf types. This fact made possible construction of growth diagrams based upon the relative growth constants as determined in figures 6 to 12.

These growth diagrams were made by projecting, at the proper angles, from a midpoint corresponding to the point of petiole attachment, lines representing the veins and deepest point of indentation of the sinuses. Then taking a given value for B from the growth curves, the length of each of the other projection lines at the time B equalled the given value was marked. Connecting lines were drawn between these points.

Figures 13, 14, and 15 show these diagrams for the three principal leaf types. Each diagram represents, by virtue of the method used, the average form of twenty-five leaves of a given genotype at various stages. It is readily apparent that they give a true picture of shape changes of each type, but two facts must be kept in mind when they are compared with figures 2, 3, and 4. First, the drawings for figures 2, 3, and 4 were made from leaves selected early in the experiment as typical of each genotype; and second, there are most certainly small growth differences, particularly around the margins and at the bases of the leaves within each type, some doubtless due to genes other than the two with which we are primarily concerned, and some perhaps environmental.

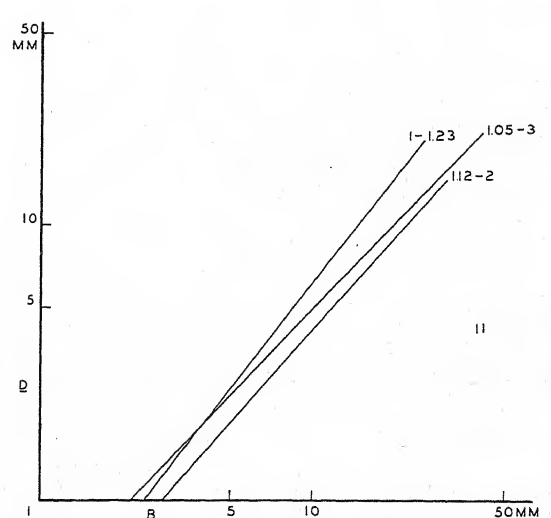
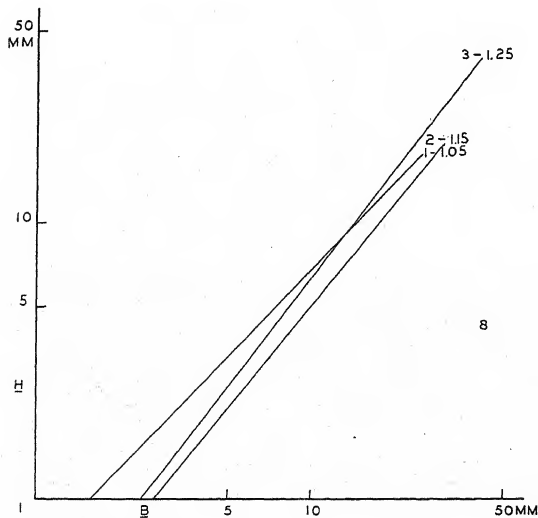
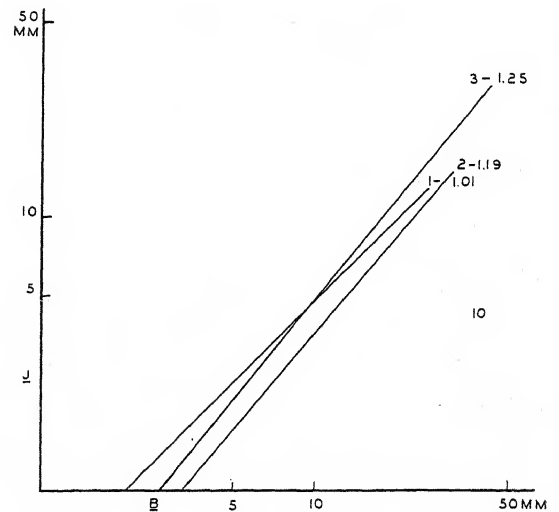
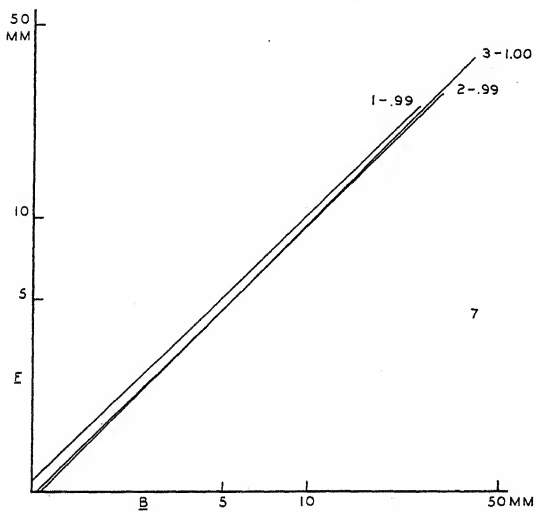
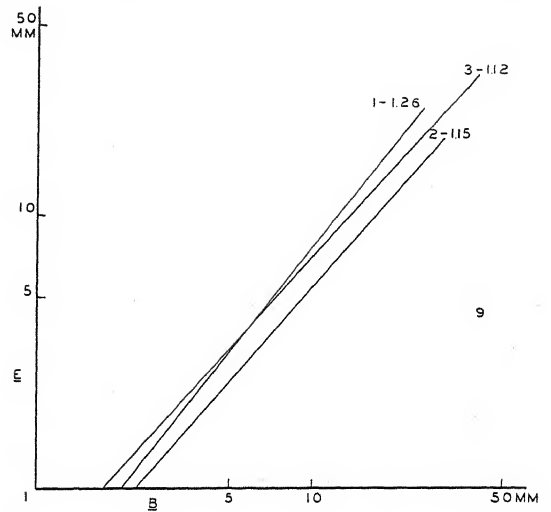
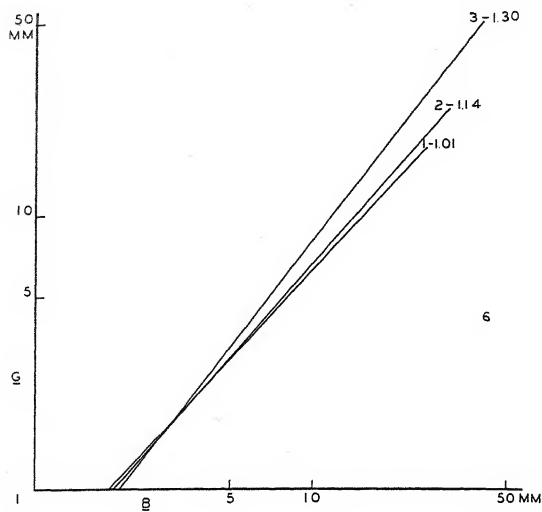


Fig. 6-11. Growth of the lobes and sinuses plotted against growth along the midrib. Plotted on double logarithmic paper.—Fig. 6. Growth of sinus G (fig. 5) against midrib B. 1, acutely lobed leaf; 2, roundly lobed leaf; 3, orbicular leaf.—Fig. 7. Lobe F against B.—Fig. 8. Sinus H against B.—Fig. 9. Lobe E against B.—Fig. 10. Sinus J against B.—Fig. 11. Lobe D against B.

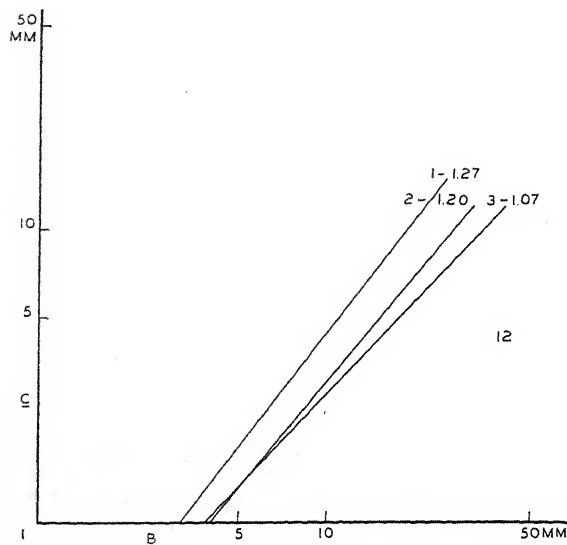


Fig. 12. Growth of the lobes and sinuses plotted against growth along the midrib. Plotted on double logarithmic paper. Base C against B.

Considering first the smallest outlines in each of the diagrams (fig. 13, 14, and 15), which depict the shape of the leaves when line B is equal to 2.5 mm., it will be seen that all three leaves are identical in shape. Progressing toward the margin of the leaf, the next outline shows the shape at a length of 5.0 mm. (line B). At this stage the two lobed leaves are still very similar, but the sinuses of the orbicular leaf have begun to be obliterated by the more rapid extension of tissue, particularly along line G. At the next stage (B 10 mm.) the sinuses are still less apparent in the orbicular leaves, and their depth is perceptibly less in the roundly-lobed type. In the fourth outline (B 20 mm.) the sinuses have completely disappeared in the orbicular leaf, are still more shal-

low in the roundly lobed leaf, but remain relatively the same as earlier in the acutely lobed form.

The acutely lobed leaves mature, and growth ceases when B is approximately 25 mm. long, and their shape is, except for some minor adjustments, about the same as that of the juvenile leaf. The roundly lobed leaf grows somewhat longer, until B is 30 mm. in length. As seen in figures 6, 8, and 10, the sinuses here are growing about 1.15 times as rapidly as the lobes and the result is a partial obliteration of the lobes. What was originally selected as the orbicular leaf type is now known to have included plants of two different genotypes (*Lu* and *LU*) in which the relative rates of growth are approximately the same during the greater part of development, but which ultimately bear leaves of slightly different shape. Unfortunately, these types were not segregated in the original measurements. However, the evidence is sufficiently clear as to leave no doubt concerning the conclusions.

In the first of these (*Lu*), growth ceases when line B is approximately 35 mm. in length (the dotted line in fig. 15). At this point the sinuses, by their considerably more rapid growth, have just about overtaken the lobes, and the leaf is almost circular in outline. In the other type growth continues longer, usually until B equals 40 mm. or more. By the time this stage is reached the tissue of the sinuses, particularly G and H, has been extended considerably beyond the margins of the lobes. When this happens, the margins of the leaves either curl up in these areas or the whole periphery becomes wavy. In no case does the area which once was sinus project as a lobe, appar-

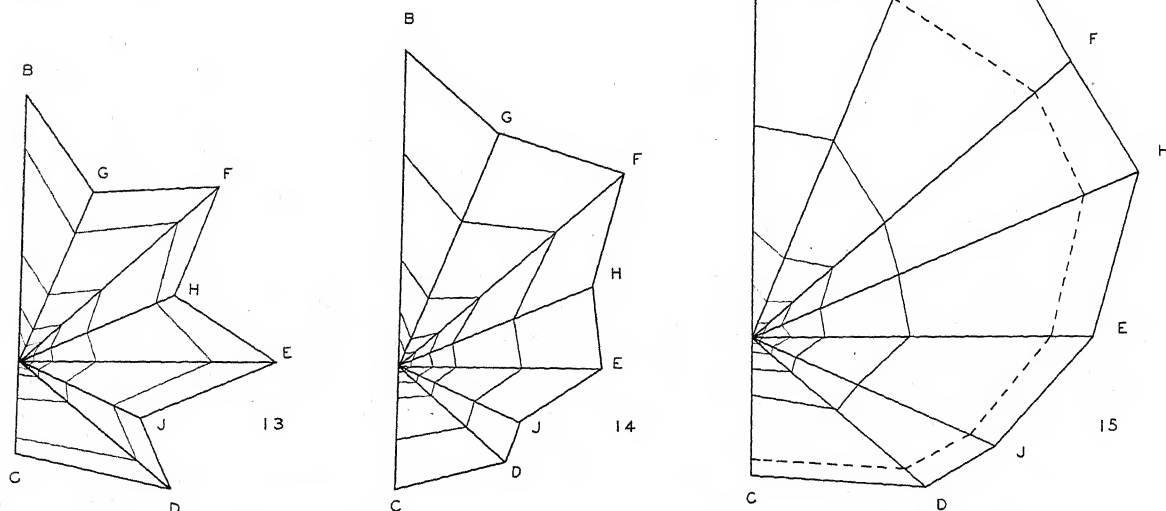


Fig. 13-15. Diagrams of progressive growth stages. See text for explanation.—Fig. 13. Acutely lobed leaf *lu*.—Fig. 14. Roundly lobed leaf *lU*.—Fig. 15. Orbicular leaf *Lu* and *LU*.

ently because of a lack of rigidity resulting from the absence of large veins.

It is also worthy of note that in all the leaves growth along line E is more rapid than growth in the terminal lobe. This, of course, makes the mature leaves wider with respect to length than the young leaves. Relatively, too, there is much stronger growth in the basal part of the lobed leaves, particularly in the acute one. While these facts are not so important in determining final shapes, they may be of value in any attempt to explain the mechanism.

Developmentally there appear to be, then, two major considerations involved in the determination of leaf patterns here. First, there are differences in the relative rates of growth of lobes and sinuses. In the acutely lobed leaf, which is known to be of the genetic constitution *lu*, the lobes and sinuses grow at about the same rate and the original juvenile pattern is maintained. In the roundly lobed leaves, *lU*, the sinuses grow somewhat faster than the lobes and thus tend to lose their identity. In the orbicular leaves, *LU* and *LU*, the sinuses grow very much more rapidly than the lobes. Secondly, there is a difference in the absolute size of the leaves at maturity (table 1). The acutely lobed leaves are smallest, the

TABLE 1. Measurements of mature *Tropaeolum* leaves (from Whaley, 1939).

Genotype	Leaf size, average of 100 leaves	
	Length (cm.)	Width (cm.)
<i>LU</i>	7.49±.09 ^a	8.32±.15
<i>Lu</i>	6.69±.09	7.29±.11
<i>lU</i>	5.59±.07	5.25±.06
<i>lu</i>	4.27±.12	4.63±.10

^a Standard error.

roundly lobed ones somewhat larger, and the orbicular ones of two sizes depending on their genotype, *LU* being larger. Obviously these two factors are correlated, for the leaf with the genetic constitution *LU* not only has more rapidly growing sinus tissue, but is larger, absolutely, than those leaves which are genetically *lu*. Likewise, leaves which are *Lu* not only grow larger, but their sinus tissue grows even more rapidly than that of the *lU* leaves. The presence of *L* and *U* together in the same plant does not, on the other hand, appear to affect the sinus/lobe growth any further, but does seem to produce greater absolute growth.

The leaf primordium in *Tropaeolum* arises from the apical meristem as a simple protuberance. This first outgrowth is the portion which is later to comprise the terminal lobe. It is flanked as growth proceeds by successive pairs of protuberances which eventually form the other lobes of the leaf. As Smirnov and Zhelochovtsev (1931) have shown, the very young leaf contains sharp deep sinuses. In all species whose leaves resemble those of *T. majus* the origin of the leaves appears to be similar, and the juvenile leaves are of the same general form.

Shape differences here, as the foregoing evidence attests, are due to differences in relative dimensional growth rates which appear fairly late in development. They are of the same general type as those found by Sinnott and Kaiser (1934) in the developing fruits of *Capsicum* as opposed to those which the same authors found in *Cucurbita* where the various shapes are visible in the earliest primordia and persist unchanged until the fruit matures.

In this instance, however, a further analysis has made it possible to obtain some idea of the manner in which these shape differentiations operate. Von Papen (1935) studying the growth of the leaf blade in *Tropaeolum* made a series of determinations of the number of epidermal cells per unit area of surface in different parts of the leaf at various stages of development. She found that in mature leaves there is a fairly even distribution, indicating that all the epidermal cells are of approximately the same size. Making counts on younger leaves, she determined that the number of cells was proportionately very much greater (i.e., the cells were smaller) in certain areas than in others and that the amount of difference varies inversely with the age of the leaf. The regions where, in the youngest leaves, the cells are very markedly smaller than elsewhere, are precisely the regions where the present authors found greatest relative growth to take place in the roundly lobed and orbicular leaves, namely, regions G, H, E, and the basal part of the leaf.

It is significant that von Papen's counts were made upon the young leaf of a plant whose mature leaves were almost orbicular. The authors have since made several counts upon juvenile leaves of the acutely lobed type and find that, provided the areas immediately adjacent to the veins are avoided, there is no significant difference between the size of the epidermal cells at the bases of the sinuses and elsewhere in the leaf.

A series of cell measurements was made on ten mature leaves of each type, taking a hundred cells from the area between lines B and G at a point approximately 20 mm. from the petiole in each instance. The results are given in table 2. Cell size measurements in leaves, particularly leaves of the larger forms where the tissue organization is very loose, are not particularly satisfactory, and, as can be seen in table 2, the differences obtained here are not all significant. They do, however, indicate that in comparable regions the larger leaves have considerably larger cells.

The details of cell size, then, resolve themselves along two definite lines. First, in those leaves which are later to become roundly lobed or orbicular the cells in the areas of greatest potential growth (epidermal cells at least) are relatively small. Second, there are different absolute levels of mature cell size in the different types, the largest leaves having the largest cells.

The individual action of the genes is not altogether clear, but the data seem to suggest that *U* produces a greater intensity of division in certain areas of the

TABLE 2. Cell size measurements in microns.

Genotype	<i>l u</i>	<i>l U</i>	<i>L u</i>	<i>L U</i>
Upper epidermis:				
Periclinal diameter	53±4 ^a	48±4	54±6	72±7
Palisade mesophyll:				
Anticlinal diameter	60±3	67±7	88±10	104±5
Periclinal diameter	12±0.3	14±0.8	12±0.9	17±0.4

^a Standard error.

leaf very early during development. It appears also to increase the absolute mature size of the cells. *L* also has the effect of producing areas of smaller cells, cells with greater expansion potencies, but at the same time it produces considerably more cell growth than *U*. When *L* and *U* are present together there is some additive effect resulting in even greater cell expansion.

Thus the shape pattern is determined during the period of division very early in ontogeny, although no shape differences are distinguishable in the young leaves (1-2 mm. stage). The ultimate form of the leaf is controlled to a large extent by the amount of cell expansion. Either by longer continued or more rapid division in certain regions than in others a definite pattern consisting of differences in the number of cells per unit area in different regions is laid down in this very early stage. Later during the period of cell expansion, all of the cells of a given tissue tend to expand to approximately the same size. As a result those parts of the leaf which contain more cells per unit area grow more than the other regions. Thus the ultimate shape of the leaf is controlled by the extent

to which cell expansion develops the original pattern laid down by differential cell division.

SUMMARY

Leaf shape in a species cross of *Tropaeolum majus* var. Golden Gleam × *T. peltophorum* var. *fimbriatum* is controlled principally by two genes *L-l* and *U-u*. Evidence is presented to show that *U* produces greater intensity of division in certain areas of the leaf very early in development. *L* has the same effect but to a lesser extent. The main effect of *L* is to produce considerably greater cell growth than *U*. *L* and *U* together result in the greatest amount of cell expansion.

The shape pattern is laid down by differential cell division early in development. The final shape of the leaf is determined by the extent to which cell enlargement develops the potential patterns.

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ON THE NASTIC AND TRAUMATIC RESPONSES IN THE PEA TEST¹

Charles L. Schneider

THE "PEA TEST" of Went (1934), a test method for the plant growth hormone auxin, depends on the curvature of longitudinally slit, growing stems (see sketch, fig. 1). Because of released tissue tensions and compressions, the slit halves of the stems curve strongly outward, a new equilibrium position being reached after a short time. In auxin solutions the preliminary outward curvature is soon followed by an inward curvature, *i.e.*, a curvature toward the cut surface. This inward curvature is a result of unequal growth of the "inside" and "outside" layers of tissue and is a function of the auxin concentration.

There has been a series of investigations on the physiological mechanism of this response (Went, 1934; Jost and Reiss, 1936; van Overbeek and Went, 1937; Thimann and Schneider, 1938, 1939; Jost, 1938; Went, 1939). The explanations offered cover most of the conceivable possibilities and include: 1. Growth of the layers of cells near the longitudinally cut surface such that these "inner" cells become more nearly isodiametric; *i.e.*, they decrease in length although they increase in volume. Thus the "inside" becomes shorter and causes an inward curvature. 2. Auxin can enter only through the intact surface (not through the cut surface), and establishes, therefore, an auxin gradient such that the outer layers are stimulated to grow more than the inner. 3. All layers have about equal access to the supplied auxin, but the central and peripheral layers inherently accumulate auxins to different degrees or respond to them to different degrees—*i.e.*, the response is nastic. 4. The injury resulting from slitting reduces the potential growth of cells near the cut—*i.e.*, the response is traumatic.

The first two explanations have already been abandoned. The nastic and traumatic explanations remain; their evaluation in relation to each other is the subject of this paper.

In making this evaluation, allowances must be made for the variable condition of the material used. For example, the finding that a given fraction of the curvature of highly responsive halved stems is the result of trauma or of nastic need not necessarily hold exactly in another case where only slightly responsive halved stems are encountered. In this connection it is of interest that with pea stems the reactivity varies to such a degree as to give the large curvatures reported by Went (1934, 1939) or no inward curvatures at all as reported by Jost and Reiss (1936); similarly with dandelion (*Taraxacum*) flower stalks, either inward pea test curvatures or only extreme outward curvatures can be obtained, in this case, de-

pending on the pre-treatment of the stalks (Jost and Reiss, 1936).

Certain artificially varied conditions of the experimental material are most useful as, *e.g.*, in the analysis below, since they make possible the consideration of each variable separately.

EXPERIMENTAL CONDITIONS AND MATERIALS.—The experiments reported here were performed with plants grown in a dark room at about 25°C. and 80 to 90 per cent relative humidity. Red and orange light only was used for illumination. Where not otherwise stated, the auxin used was 3-indole-acetic acid; solutions were made up in distilled water; the optimum concentration for curvatures with coleoptiles is about 5 mg./l., for stems about 10 mg./l.; no nutrients were added.

Seedlings of peas, *Pisum sativum*, var. Alaska, and of oats, *Avena sativa*, var. Victory, were used. Young oat coleoptiles (from three-day-old seedlings) and the growing parts of pea stems (from seven-day-old seedlings) were used. Coleoptiles were decapitated and slit for a distance of 1.5 cm. beginning at the cut surface; stems were decapitated and slit 2 cm.

THE NASTIC EXPLANATION.—Investigators supporting the nastic explanation have found it necessary to make appropriate experimental balances for tendencies to curve, caused by trauma. This can be done in several ways.

Balancing out the trauma gradient by injury on all sides.—The "inside" injury gradient, caused by slitting the stem, can be counterbalanced by injuring the outside also, as, *e.g.*, by peeling off the epidermis (Thimann and Schneider, 1938; *cf.* van Overbeek and Went, 1937). When this is done, inward curvatures are still obtained. Since all sides are injured, it is simplest to conclude that the curvatures are nastic.

Balanced trauma gradient in a perpendicular plane.—Another way of balancing out trauma effects is by slitting longitudinal ribbons from coleoptiles (fig. 2) of *Zea Mays* (favored because of large diameter, fig. 6) or of *Avena* (Thimann and Schneider, 1939). The inner and outer epidermal layers of these ribbons are thus both left intact. The injury gradients not only balance each other but are perpendicular to the plane of curvature and hence can hardly contribute to the curvature.

However, the cross sectional form of these ribbons changes during the test. The change is in a direction such that the injured surfaces have an inward component; *i.e.*, they take on the shape of the "quarters" of figure 2 (*cf.* Went, who reports the absence of such curvatures, 1939). Presumably this results from an excess lateral growth of the outer layers of the ribbons and hence would be, in itself, a nastic response, and thus is more a support of than a contradiction to the nastic explanation (Thimann and Schneider, 1939). The possibility of inward curvatures arising from this component of trauma can, however, be

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eliminated from the experiment by using "corrected" ribbons such as shown in figure 2. As these ribbons grow, the cut surfaces, which at first have an outwardly directed injury component, became parallel, but inward pea test curvatures are still obtained (Thimann and Schneider, 1939).

Thus whether the injury is balanced out by injury of all surfaces or by appropriate distribution of injured surfaces, strong inward curvatures are still obtained; these curvatures must be largely nastic in origin.

However, the removal of epidermis does more than merely cause injury; it also removes one of the important structural components of the organ. The restraining influence which the epidermis normally exerts on elongation is so great that, upon its removal, inward curvatures are apt to be obtained even in water controls (cf. Went, 1939). Still larger inward curvatures are obtained in auxin solutions (cf. Thimann and Schneider, 1939). It is to be noted that in this particular case, the nastic response must have been strong enough to more than overcome the op-

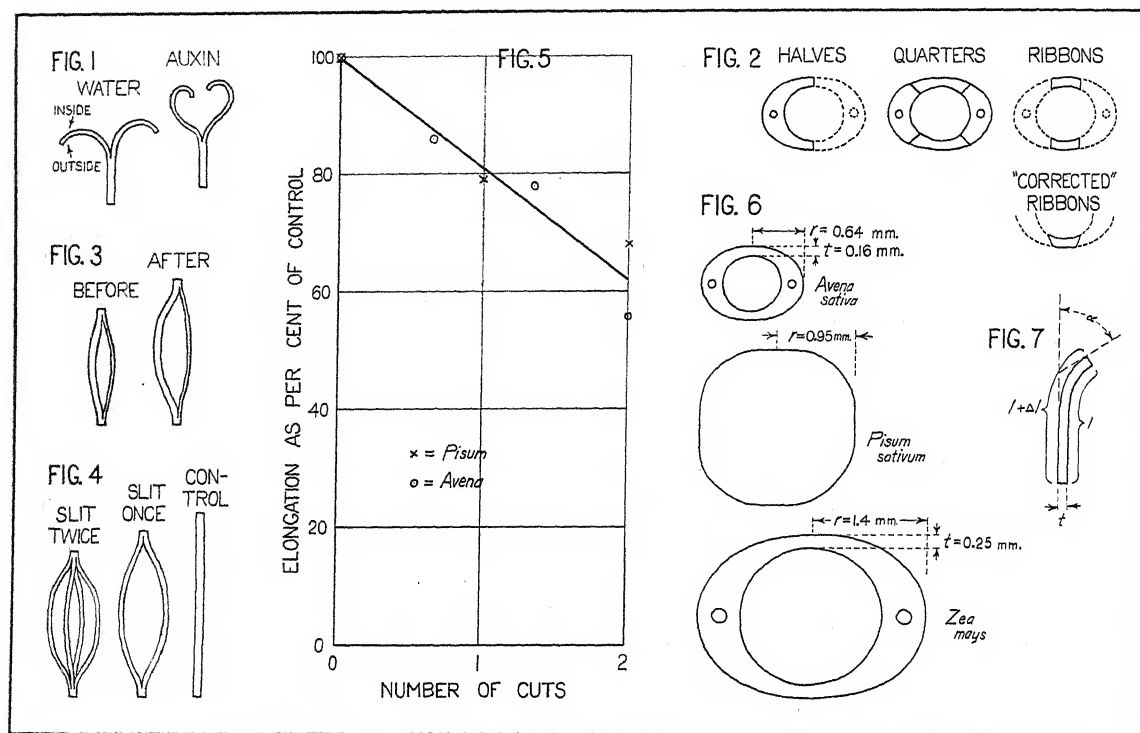


Fig. 1. Curvature of longitudinally slit (halved) *Pisum* stems in water and in auxin solution.
 Fig. 2. Cross section of coleoptiles showing: halves, quarters, ribbons and corrected ribbons.
 Fig. 3. *Pisum* stem segments slit asymmetrically almost to the ends before and after treatment with auxin.
 Fig. 4. Segments grown in auxin after slitting once (halved) or twice (quartered).
 Fig. 5. Elongation in auxin solution of segments of *Pisum* stem and of *Avena* coleoptiles when slit once or twice (as in fig. 4).
 Fig. 6. Dimensions of cross sections of *Avena sativa* coleoptile, *Pisum sativum* stem and *Zea mays* coleoptile.
 Fig. 7. Diagram for calculating relation between curvature and differential increase in length.

Reversing the trauma gradient.—An even more striking demonstration that curvature can be caused by a nastic response is obtained by reversing the trauma gradient. To do this, hollow organs such as coleoptiles of *Avena* (Thimann and Schneider, 1938) and flower stalks of *Taraxacum* (Jost, 1938) peeled on the outside can be used. Since a large portion of the "inner" surface of these tubular organs is still covered by the inner epidermis after slitting, the inside is relatively intact and the greater injury, when the organs are peeled, is on the outside; hence, the trauma gradient would tend to cause curvature in the outward direction. Yet these objects give inward curvatures. These curvatures must be of nastic origin.

positely directed tendency for a trauma response. Because of the intimacy of injury with all the remaining tissue and, therefore, greatly reduced growth, this does not provide evidence that nastic is the most effective cause of curvature in the more general case; however, it does provide a special case in which curvature must be accounted for by the nastic response.

A DIGRESSION ON OUTWARD CURVATURES.—Increased outward curvatures obtained with appropriate concentrations of auxins, especially 3-benzofuran-acetic acid, have been considered to be a result of, and support for, the nastic interpretation by Thimann and Schneider (1938). That these curvatures need to be reconsidered, follows from the data pre-

sented in table 1. In this experiment (confirmed by a second experiment), the curvatures of both stems and coleoptiles are compared when peeled and when not peeled for serial dilutions of benzofurane-acetic

TABLE 1. Curvatures of peeled and of ordinary (i.e., not peeled), halved stems and coleoptiles in 3-benzofurane-acetic acid. Measured by stem reference method.

Degrees curvature of	3-benzofurane-acetic acid, mg./l.				
	0	0.08	0.4	2	10
Halved <i>Pisum</i> stems:					
Not peeled	-120	-140	-220	-290	+ 70
Peeled	-260	-270	-120	- 80	+ 10
Halved <i>Avena</i> coleoptiles:					
Not peeled	- 10	- 20	- 20	-100	-163
Peeled	+120	+150	+140	+130	+190

acid. (The benzofurane-acetic acid crystals were part of the same sample as used by Thimann and Schneider, 1938.) If the increased outward curvatures of ordinary halved organs were nastic in origin, similar increases in outward curvature would be expected with the peeled halves. As can be seen from the table no such trend was found. Perhaps, rather, the outward curvature obtained with not-peeled halves in solutions of this auxin or in low concentrations of other auxins is due to easier entry of auxin on the injured side. Whether this be the correct explanation or not, the outward curvatures obtained with this auxin can no longer be considered as adequate support, in themselves, for the nastic explanation.

THE TRAUMATIC EXPLANATION.—For investigators supporting the nastic explanation, no ways have been found for completely eliminating or balancing out the nastic response. Whenever a trauma gradient is produced, a nastic gradient is also produced because of affecting at least one layer of tissue to produce the trauma. For experimental investigation, however, peeling the epidermis off one side of a pea stem (Went, 1939) approaches this state closely, and inward curvatures are obtained in auxins (*cf.* also Thimann and Schneider, 1938, for the *Avena* coleoptile). Since the organ is structurally still nearly symmetrical when the epidermis is thus asymmetrically removed, the curvature produced must be largely due to the trauma gradient.

Decrease of growth by injury.—In favor of Went's trauma explanation (1939) is his observation that longitudinally slit stems grow less in response to auxin than do stems that are not slit. This reduction of potential growth by injury and the reasonable assumption (backed up by deductions from experiments) that there is a trauma gradient away from the site of injury, led Went (1939) to the conclusion that injury is responsible for auxin curvatures in the pea test.

Additional evidence for this view is provided by the measurements of van Overbeek and Went (1937;

cf. Went, 1939). In optimal concentrations of auxin, the "outside" layers of halved *Pisum* stems grew the same amount as intact control stems, but the tissue near the slit surface grew much less. Although this difference in growth between the layers farthest from and those nearest to the slit may be partly explained by nastic responses, the data are highly consistent with the postulate of a trauma gradient directed away from the site of injury.

Further, by determining the actual elongation of inside and outside, Went (1939) was able to show that the responsiveness of tissue near the wound decreased more rapidly with time after making the wound than did the responsiveness of tissues farthest from the wound. This trauma effect is complex, the more apical regions responding differently than the older regions below (Went, 1939).

Decreased responsiveness to auxin as a function of amount of injury.—That relatively more injury causes greater decrease of growth can be demonstrated by longitudinally slitting a segment of stem to one side of the axis, with the slit not extending quite to the ends of the segment (fig. 3). This gives two strips, one thick and one thin, attached to each other at both ends. Both strips have the same absolute injury, since they are from opposite sides of the same cut. On allowing growth to proceed, especially in optimal concentrations of auxin, the result is a bow shaped object (obtained repeatedly) with the thinner strip forming the string of the bow (fig. 3). According to the nastic explanation the thinner strip should have grown most, since it is largely "outer" tissue. That the thin strip grew least must be interpreted to mean that the trauma effect is large enough to more than offset the nastic effect.

Quantitative relations between growth and amount of injury.—Stem and coleoptile sections of known length (2.0 cm. for stems and 1.5 cm. for coleoptiles) were slit as shown in figure 4 and placed in optimal concentrations of auxin (5 mg./l.). By leaving unslit portions at both ends, the sections remained fairly straight and could be further straightened beside a rule to estimate the amount of elongation. The injury can be increased by slitting a second time in the perpendicular plane. For the coleoptiles, the amount of injury can be still further varied by making the single slit either in the plane of the vascular bundles where the coleoptile is thickest (*cf.* fig. 6) or in the plane at right angles to this where the coleoptile is thinnest. (In figure 5, coleoptiles slit in these two ways are plotted as 4/3 and as 2/3 of a cut respectively.) The resulting effects of injury on the straight growth in auxin are plotted in figure 5. The elongation for the injured sections is, in each case, plotted as percentage of the elongation of the controls. The actual elongation of the *Pisum* controls was 40 per cent and of *Avena* 26 per cent over the original lengths. The results are as though, under the conditions investigated, the decrease of potential growth by trauma, for a given volume of tissues, is approximately proportional to the area of injury (fig. 5).

Trauma index for coleoptile and stem.—The growth decrease relative to the amount of injury and tissue involved can be designated as the trauma index. Its value is measured by the slope of the curve of figure 5; it is about the same for *Avena* coleoptiles as for *Pisum* stems under these conditions of etiolation.

SIMULTANEOUS EFFECT OF NASTY AND TRAUMA.—In summarizing the above evidence, it is to be seen that there need be no mutual contradiction of the nastic and traumatic explanations. Thus:

In certain special cases where the injury gradient is balanced out or reversed, the curvature of slit stems must be almost entirely nastic.

Under the conditions of the pea test, trauma reduces the ability of tissues to grow, and it appears that this trauma effect has a gradient away from the site of injury; therefore, in cases where injury is asymmetrical, trauma should tend to cause curvature. In one special case the nastic effect can be largely balanced out; the curvature in this case must be largely traumatic.

Since nastic and traumatic responses may be considered as experimentally established and as supplementary rather than contradictory, the pea test problem becomes one of estimating the relative importance of the nastic and traumatic responses.

NASTIC RESPONSE AS THE STANDARD FOR COMPARISON FOR THE RATIO, NASTY/TRAUMA.—The special cases (above), in which curvature is entirely or predominantly nastic, can be used to estimate what fraction of the curvature of the more general cases is nastic. The evaluation will depend on setting an upper limit to the ratio of effectiveness for nasty/trauma. An exact evaluation is not possible here, both because of the complex mathematical analysis and because of complicating biological factors. Where uncertainty arises, favor is shown toward nasty; in the end this gives small error, because, at best, for the general case, nasty turns out to be the lesser cause of the curvature.

Comparison of ribbons and of halves of coleoptiles.—If we consider that the portions of halved coleoptiles near the slit are the equivalent of "inner" tissue and that these grade into "outer" tissue at the middle of the semi-circular arc (see fig. 2), then the distance separating inner and outer tissue, in the plane of curvature is the distance r , the radius of the coleoptile. In the ribbons, inner and outer tissue are separated by not more than the distance t , i.e., the thickness of the coleoptile tissue in the region from which the ribbon was taken (see fig. 6).

The mathematical analysis comparing the curvatures of these halves and ribbons is analogous to that for the curving of a bi-metallic strip (ribbon) as compared with the curving of a bi-metallic half-cylinder made by inrolling of the strip. The same geometric relations that allow only a small curvature of the bi-metallic half-cylinder should allow only a small curvature of the halved coleoptile. For the present, however, both the ribbon and the half-cylinder will be considered as simple, strip-like structures grad-

ing from "inner" to "outer" layers. While this assumption does not permit an exact analysis, it makes possible the estimation of an upper limit.

The curvature caused by unequal extension of the "inner" and "outer" layers can be calculated by the formula: $a = k \times \Delta l / t$, where a is the angle of curvature, k is a constant, Δl is the differential increase in length of the "inside" and "outside," and t the distance between the inner and outer layers (fig. 7) (see Purdy, 1921).

Using this formula to deduce the ratio of curvature to be expected for halved coleoptiles and for ribbons, we find the ratio to be t/r . Actual measurements of t and r for *Avena* (fig. 6) give a ratio of the order of 1:4 (0.16/0.64). Hence, if the nastic influence were also the *only* cause of curvature in the halves (as in the ribbons), the ratio of curvatures of halves and ribbons should be 1:4. On the contrary, the relative curvature for quartered against halved coleoptiles is about 1:1; i.e., the halves curve about four times as much as could be accounted for on the basis of nastic responses alone; i.e., only about 0.3 (one-fourth) of their curvature can be accounted for as nastic. The remaining 0.7 must be accounted for as traumatic.

In obtaining this ratio, quartered coleoptiles are considered the equal of ribbons. This is not strictly true, for quarters curve more than ribbons (averages of 180° as against 160° in one experiment, 180° as against 60° in another), but the deviation is in the direction to give the nastic view the advantage and, therefore, can be used in setting an upper limit to the importance of the nastic effect. Using this assumption, the ratio 1:1 can be deduced from figure 3 of Thimann and Schneider (1939); its actual value depending on the choice of control values for the quarters. It might be argued that taking the total differences between water controls and optimal response in this figure would give a value of 1:2. Even on such a basis, only 0.5 of the curvature could be accounted for as nastic, but this comparison would be misleading because the halves are prevented by their geometric structure from giving as large outward curvatures as the quarters; the epidermis especially tends to restrict outward curvatures of the halves. (For similar restrictions in the case of halved pea stems, see results of Thimann and Schneider, p. 625, 1939. It is shown there that the tension of the epidermis near the cut surface prevents full extension of the "inner" layer, thus limiting the magnitude of the outward curvature.) Thus the lower half of the curve for quarters is mechanically impossible for the halves, although physiologically it is to be predicted. Fortunately, the upper parts of the curve for halves and quarters coincide so that this mechanical difficulty can be eliminated from the calculations by using as control value for both halves and quarters either zero curvature or the control value of the halves. This gives the ratio quoted above—about 1:1.

It will be noted that the original assumption above gives the nastic explanation much advantage in this discussion. Thus for the halved coleoptiles, the tissue

nearest the cut has been considered as "inner" tissue and the tissue farthest from the cut has been considered as "outer" tissue. But, because of its component of outer tissue, the "inside" of halved coleoptiles would tend to grow more than would purely inner tissue and, conversely, the "outside," because of its component of morphologically inner tissue, would tend to grow less than would purely outer tissue. Taking this added advantage for the nastic explanation into account, the expected ratio of t/r for the postulated case of no trauma response in the halved coleoptile would be effectively smaller than 1:4; hence, even less than one-fourth of the curvature could be accounted for by the nastic response.

This analysis ascribes so large a rôle to the trauma response as to make independent analysis from other sources of great interest.

Trauma in the curvatures of slit coleoptiles as well as in slit stems.—Besides the measurements plotted in figure 5 which show that trauma affects growth of sections of *Avena* coleoptiles and of *Pisum* stems to the same degree, it follows from a consideration of geometric structure that trauma should play about as important a rôle in the curvature of halved *Avena* coleoptiles as in that of halved *Pisum* stems. This contradicts the contention of Thimann and Schneider (1938) that injury is unimportant in the curvatures of halved coleoptiles because of the small amount of injury involved. The injured area of the coleoptile is small, but so is the amount of tissue.

For the halved coleoptile the tissue farthest from the slit is at about the same distance from the site of injury as in the halved pea stem. Thus the shortest distance, *through coleoptile tissue*, from the slit to the farthest "outside" tissue is a little less than 1 mm. (fig. 6: $2\pi r/4 = \pi 0.64/2 = 1$ mm.). In the halved pea stem the shortest distance (the radius of the stem) is also about 1 mm. Thus the same geometric structure that reduces the absolute amount of injury for the halved *Avena* coleoptile assures distribution of injury in such a manner as to compare physiologically with that of the halved pea stem.

Because of the smaller radius of the coleoptile, other things being equal, this results in an even steeper component of trauma gradient in the plane of curvature for the coleoptile than for the stem. Therefore, although there is less absolute injury upon slitting a coleoptile than upon slitting a stem, the effect of trauma on subsequent curvatures can be at least as large, and the trauma explanation of curvatures is certainly no less appropriate for halved coleoptiles than for halved stems.

Peeled vs. not-peeled halves of stems.—A similar comparison can still be made by considering the curvature of peeled halved pea stems as nastic in origin and that of ordinary halves as nastic plus traumatic in origin. The data of Thimann and Schneider (fig. 2A, 1938) obtained by the "inflection reference" method is suitable for this purpose. The inward curvature of peeled halves in auxin is 100° , that of ordinary halves 250° . Presumably, the increase of 150° is the result of the trauma gradient; hence, the ratio

of nasty/trauma is $1/1.5$; i.e., the curvature of halved pea stems would be $2/5$ (i.e., 0.4) nastic and $3/5$ (i.e., 0.6) traumatic in origin. As seen below this too probably favors the nastic interpretation.

As in the comparison of ribbons (or quarters) and halves of coleoptiles above, the differences of the structure of the curving elements becomes important. The outward curvature of ordinary halves is greatly restrained by the epidermis as compared with that of the peeled halves (cf. Thimann and Schneider, p. 625, 1938), and direct comparison of water controls of peeled and ordinary halves by the "stem reference" method is thus made impossible. Measurements based on the inflection reference method eliminate that part of the curve which is structurally impossible for the ordinary halves to attain.

Just as removal of the epidermis allows an increase of outward curvatures of water controls, it may also allow increase of inward curvatures in the auxin treatment. This would place the value of 0.6 obtained above, for the traumatic effect as a minimal estimate.

TRAUMA RESPONSE AS STANDARD FOR COMPARISON FOR THE RATIO, NASTY/TRAUMA.—In all of the above cases, the analysis has depended on comparison of curvatures with a special case in which the cause of curvature was entirely or predominantly nastic. The fraction of the curvature that could not be accounted for as nastic in the more general case was then concluded to be of trauma origin. The over-all findings indicate that probably less than one-half of the curvature of ordinary halved organs in the pea test is nastic; the remainder, more than one-half, is concluded to be traumatic. To check this conclusion it is desirable to make a similar comparison in a case in which the nastic response is largely balanced out and trauma is the predominant cause of curvature. The case most nearly approaching balancing out of nasty but having trauma is that in which the epidermis is peeled off one side of an (whole, i.e., not slit) organ. In this case, the tissues, with the exception of the epidermis, are so symmetrically disposed as to balance out nearly all of the nastic tendencies for curvature. The resulting curvatures must be predominantly of trauma origin.

Making use of the formula of Purdy, quoted above, that fraction of the curvature of ordinary halves which can be accounted for as traumatic, can be estimated by comparison with not-slit organs which are peeled on one side. If it be assumed, among other things, that in both cases the tissue near the wound is strongly affected by trauma and that the tissue farthest from the wound is free of trauma effect, it would be expected that the halves would curve twice as much from trauma alone as would the not-halved organs which are peeled on one side. Data on this point for *Pisum* stems are available from Went (1939, fig. 3). The ratio is actually $1.5/1$ (c. 330° to 220°). Similar data for coleoptiles are available for *Avena* from the experiments of Thimann and Schneider (1938, fig. 8 and 3). In this case the ratio is $1.6/1$ (c. 420° to c. 260°). If these data were used directly, it should be concluded that at least all of

the curvature of ordinary halves is of trauma origin because, by deduction from the formula of Purdy, a ratio of greater than 2/1 would be required before any of the curvature of the ordinary halves would be left to be accounted for by the nastic effect. However, it will be noted that a direct comparison is not valid because of the shape of the cross section of the organs that are peeled on one side. If the organs are peeled on half of their circumference, then the wound area is not localized at a distance of a full diameter from the intact side; much of it is closer. This effectively reduces the ratio of curvatures to be expected on the basis of trauma alone and favors the nastic explanation; the lowest value that could be expected would be 1/1 if the trauma effect from the peeling extended so far around as to be almost as effectively distributed as in the ordinary halves. Using this extreme assumption, the ratios of 1.5/1 and 1.6/1 would indicate that about 0.7 of the curvature of halved stems and 0.6 of the curvature of halved coleoptiles is of trauma origin. The remaining fractions, 0.3 and 0.4, would then be concluded to be of nastic origin. This distribution agrees well with the estimates obtained above, in which comparison was made with special cases wherein the nastic response was the predominant cause of curvature.

CONCLUSION.—In comparing the effectiveness of the two causes of curvature in the pea test, only the most pertinent evidence for the relative evaluations have been considered. In the literature referred to are many further data and deductions of greater detail.

The conclusions arrived at here on relative effectiveness of the nastic and the traumatic responses in the ordinary pea test are essentially for optimal auxin treatment since the evaluations depend on the difference between curvatures of halves in water and in auxin solutions of concentrations in which optimal responses are obtained.

The deductions are applicable to the slit halves of organs used in the pea test and are only indirectly applicable to the growth of intact organs.

For the ordinary pea test, whether performed with slit stems or coleoptiles, the above evidence indicates that less than half of the curvature is of nastic origin, more than half of traumatic origin. The results are accumulated in table 2.

TABLE 2. *Fractions of curvature caused by nastic and by traumatic responses in the pea test. Values favor the nastic explanation and hence set an upper limit for the nastic response.*

	Nastic	Traumatic
When standard for comparison is of nastic origin:		
Coleoptiles of <i>Avena</i> :		
Ribbons vs. halves.....	0.3	0.7
Stems of <i>Pisum</i> :		
Peeled vs. not peeled halves	0.4	0.6
When standard for comparison is of trauma origin:		
Coleoptiles of <i>Avena</i> :		
Thick vs. ordinary halves...	0.4	0.6
Stems of <i>Pisum</i> :		
Thick vs. ordinary halves..	0.3	0.7

SUMMARY

The chief points establishing the nastic and traumatic causes of the curvatures of the pea test are summarized. These involve special cases wherein the curvature is predominantly either nastic or traumatic in origin together with demonstrations of a trauma effect.

The nastic and traumatic responses to auxin in the pea test are considered as complementary rather than contradictory.

From a direct measurement of the effect of injury on growth during optimal treatment with auxin, it was found that growth is affected to about the same extent in both *Pisum* stems and *Avena* coleoptiles by the same relative amounts of injury.

It was found by comparison of special cases where nastic is the only, or nearly the only, cause of curvature that values for the fraction of the curvature caused by the nastic response of ordinary halved *Avena* coleoptiles and *Pisum* stems, tend to be well below 0.5. The remainder of the curvature, more than 0.5, is concluded to be of trauma origin. These estimates are supported by a similar analysis from the opposite direction in which a largely traumatic response is used as the standard of comparison.

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THE INFLUENCE OF COPPER AND IODINE ON THE GROWTH OF AZOTOBACTER AGILE ¹

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AZOTOBACTER AGILE apparently does not require appreciable amounts of iodine in its nutrition (Lewis and Powers, 1941). The organism has been periodically subcultured over a period of one year in a special medium containing on the order of only one part per trillion (1×10^{-12} gms. per ml.) of iodine, without progressive reduction in growth-rate or maximum turbidity of the cultures. Nevertheless, iodine has been found to increase both growth-rate (Lewis and Powers, 1941) and final yield (Stoklasa, 1926; Itano and Matsuura, 1933; Greaves, 1933) of *Azotobacter* under certain conditions. It seems likely that such stimulations are due to actions other than satisfying a deficiency of an essential nutrient factor.

An observation during early experiments indicated that a stimulation of growth-rate of *Azotobacter agile* by iodine might be related to the concentrations of certain trace elements with which the culture medium was supplemented. Experiments were, therefore, conducted to determine the minimum toxic concentrations of several nutritionally important trace elements, and to test possible interactions with potassium iodide.

METHODS.—*Azotobacter agile* was inoculated into 500 ml. cotton-plugged Erlenmeyer flasks containing

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140 ml. aliquots of the experimental medium, from a two- to three-day-old subculture of the organism which had been carried for extended periods on "iodine-free" medium. Growth was measured, after it became visible, in early experiments by microkjeldahl nitrogen determinations and by plate counts, in later experiments by turbidity measurements, using a commercial photoelectric colorimeter with a wide-band filter (maximum transmission at 420 mμ). Since the effects investigated were most pronounced in the earliest visible growth stages, and did not, in general, affect crop yields, measurements in later experiments were sometimes restricted to the early growth stages.

The nutrient medium was prepared from specially purified "iodine-free" chemicals. It contained on the order of one part per trillion (1×10^{-12} gms. per ml.) of iodine. The equivalent of 1.1 per cent by weight of absolute ethyl alcohol was added after sterilization as a carbon and energy source. The pH was adjusted to 7.0 ± 0.1 . Trace elements were supplied in amounts to be mentioned later. The cultures were grown at room temperatures. Purification methods for the nutrient chemicals, the nutrient formula, and cultural details have been previously described (Lewis, 1940; Lewis and Powers, 1941).

RESULTS.—An experiment showing an effect of added iodine on the growth of *Azotobacter agile* as a function of the growth period is presented in table 1 (experiment 1). Trace elements were added at the following concentrations: Mn, 0.5; B, 0.5; Zn, 0.05; Mo, 0.05; Cu, 0.02 ppm. The effects of potassium

TABLE 1. Effect of potassium iodide on the early growth of *Azotobacter agile*; effect of stage of growth, and comparison of methods of measuring growth.^a

Iodide added (ppm.)	Experiment 1				Experiment 2	
	Concentrations of trace elements added in ppm.					
	Mn 0.5, B 0.5, Zn 0.05, Mo 0.05, Cu 0.02				Mn 0.1, B 0.1, Zn 0.01, Mo 0.01, Cu 0.004	
	Total nitrogen fixed per flask after				Growth after 2 days	
	3 days	5 days	8 days	15 days	Turbidity	Plate count
		(mg. nitrogen)			(arbitrary units) (millions per ml.)	
0	3.5±0.3	12.8±1.0	25.3±0.9	26.1±0.9	71.2±3.2	47.6±0.6
0.0001	79.0±2.8	56.6±3.1
0.001	81.5±4.0	55.1±5.4
0.01	4.7±0.2	16.7±0.7	24.7±0.6	25.0±0.5	68.8±2.4	42.8±4.0
0.1	5.3±0.2	18.7±0.8	27.1±0.2	27.3±0.2	62.3±1.2	42.5±3.3
1	5.8±0.1	17.4±0.4	26.9±0.5
50	4.9±0.4	17.2±0.2	25.1±0.6	27.0±0.4
Ave. coef. of variation (%)	9.4	7.0	3.8	3.3	7.3	13.6
Ave. growth of mature cultures				26.4±0.5	520±3 (8 days)	

^a The data of this table are means and standard errors of measurements for triplicate flasks in experiment 1, quadruplicate flasks in experiment 2.

iodide are most marked in the earliest stage, while these effects tend to disappear as the cultures attain their maximum growth. For further experiments, therefore, data will be presented for only the earlier growth stages within the range of the photoelectric colorimeter.

A second experiment (experiment 2, table 1) involved trace elements at one-fifth the concentrations used above. In this experiment, the magnitude of the iodide stimulation after two days' growth was smaller than in the preceding experiment, although still statistically significant. Also, the optimal iodide concentration was now found to be 0.0001 to 0.001 ppm. in contrast with the optimum at 0.1 to 1 ppm. in the preceding experiment.

These results prompted experiments to determine the minimum toxic levels of the trace elements with which the basal medium was supplemented. In such experiments the concentrations of trace elements were varied individually in the presence of optimal levels of the others. The following concentrations appeared to be within the optimal range and were used as basal levels in further experiments: Mn, 0.05; B, 0.5; Zn, 0.005; Mo, 0.005; Cu, 0.004; and Fe, 1 ppm. The concentrations of trace elements that gave an approximately 50 per cent reduction of growth at the stage investigated were as follows: Mn, 10; B, 25; Zn, 0.5; Mo, 10; and Cu, 0.4 ppm.

In general, a toxic concentration of a trace element either did not affect the mature yields or else prevented all growth. Cultures containing 5 ppm. of zinc showed no growth in eleven days, while 10 ppm. of iron and 2 ppm. of copper gave similar results. How-

ever, as will be mentioned later, 1 ppm. of copper in another experiment prevented growth for two weeks, after which essentially normal crop yields were attained. A small reduction in eleven-day crop yield occurred with 50 ppm. of manganese. The additions of the highest rates of iron, zinc, boron, and manganese were accompanied by precipitation.

Characteristic colors developed in the medium of the mature cultures in the presence of the higher levels of the trace elements. Molybdenum gave a bright salmon-orange color. Manganese gave a dirty pink color. Copper and iodine tended to give tan colors. Zinc cultures became very clear, while the normal cultures had a drab gray color. Iron-deficient cultures showed a characteristic pronounced greenish-yellow color. Occasionally the mature stock cultures would suddenly begin to produce a pink pigment that would finally give them a deep wine color. The first time this was observed it occurred simultaneously and to approximately the same degree in sixty different flasks that had been inoculated from the same colorless culture.

Although no special precautions were taken to obtain a basal medium low in trace elements other than iodine, a strong inhibition of growth occurred in the cases of zero and 0.1 ppm. of added iron while 1 ppm. was optimal. Likewise, zero and 0.00005 ppm. of added molybdenum were decidedly suboptimal, while 0.0005 ppm. was only slightly so. In one experiment, growth was approximately one-half maximal in the absence of added copper, while 0.001 ppm. was optimal. It should be emphasized that these deficiencies affected only the extent of growth at an early stage

TABLE 2. Determination of the minimum toxic levels of copper, zinc, manganese, molybdenum, iron, and boron during the early growth of *Azotobacter agilis*.^a

Concentration of trace element added (ppm.)	Experiment 1 (73 hrs.)		Trace element varied Experiment 2 (64 hrs.)				
	Cu	Cu	Zn	Mn	Mo	Fe	B
			(arbitrary units)				
0.0	56±12	99±6	104±4	114±4	59±6	7±1	107±12
0.00005	110±6	109±9	56±5
0.0002	94±8
0.0005	101±11	106±5	88±9
0.001	91±13
0.002	105±1
0.005	111±2	104±8	119±6	108±4
0.02	98±6	94±5*
0.05	94±5*	117±10	94±5*	94±7
0.1	105±7	28±7
0.2	41±8
0.5	39±12	55±13	94±5*	88±3	94±5*
1	14±4	94±5*
2	0
5	0	60±8	79±3	103±5
10	0
50	1±1	14±3

^a The data of this table are means and standard errors of turbidity measurements for triplicate flasks, except for means marked by asterisks which represent six flasks with trace element concentrations (Mn 0.5, B 0.5, Zn 0.05, Mo 0.05, Cu 0.02 ppm.) used except when experimentally varied, the data for which are repeated in the proper place in each column. The data are in arbitrary units; by calibration by experiment 2 of table 1, a reading of 100 is equivalent to approximately 67 million cells per ml.

TABLE 3. *Effect of potassium iodide on the toxicity of copper, manganese, and zinc in the early growth of Azotobacter agile.*^a

Iodide added (ppm.)	Experiment 1			Experiment 2		Experiment 3	
	"Optimal" trace element concentrations (31 hrs.)	Toxic Mn 10 ppm. (31 hrs.)	Toxic Cu 0.4 ppm. (31 hrs.)	Toxic Cu 0.2 ppm. (102 hrs.)	Toxic Zn 1 ppm. (84 hrs.)	Toxic Cu 0.5 ppm. (96 hrs.)	Toxic Cu 1 ppm. (25 days)
				(arbitrary units)			
0.0	88±4	42±2	20±3	32±3	37±2	71±3	2
0.01	79±4	41±3	20±2	75±14	65±3	..
0.1	82±6	38±2	39±4	96±16
1	76±5	34±3	76±3	142±25	38±2	89±8	236
10	38±2	143±3	260

^a The data of this table are means and standard errors of turbidity measurements for quadruplicate flasks. A reading of 100 is equivalent to approximately 67 million cells per ml.

and did not affect the final yields. (Data for these experiments are summarized in table 2.)

To investigate possible effects of iodine, experiments were conducted to show the effect of various concentrations of iodide in a medium containing a sufficient concentration of the trace element under consideration to give an approximately 50 per cent reduction in growth at the desired growth stage. In such solutions, the other trace elements were present at "optimal" concentrations previously given. The results of several of these experiments are given in table 3. The effect of iodine in counteracting the toxicity of copper is evident. No interactions appear to exist between iodine and manganese or zinc toxicity. Molybdenum, boron, and iron were not investigated in this respect.

An interesting effect was found in the presence of 1 ppm. of copper. These cultures, regardless of iodine treatment, showed no growth within the period ordinarily required for maturity. The cultures were saved, however, and on about the fourteenth day growth appeared in one of the cultures, which then rapidly attained the normal maximum growth. On further standing, growth appeared in the other cultures. This extended lag period was definitely shorter in the presence of iodine. The difference may be expressed on the basis of the time required for the culture to reach half-maximum growth. This period was 32.6 ± 2.5 days for the controls lacking iodine, 22.5 ± 6.0 days in the presence of 1 ppm. of iodine, and 21.2 ± 1.1 days in the presence of 10 ppm. of iodine. The data referring to this experiment in table 3 were chosen to represent a period of maximum difference between the treatments.

DISCUSSION.—It seems probable that the results found with iodine and copper were due to actions of the trace elements on the lag growth phase of the organism. This is supported by the experiment with 1 ppm. of copper in which the lag phase was greatly extended. In this case, only the lag period was altered by iodide additions, the logarithmic growth-rate was unaffected. For this reason, the sensitivity and magnitude of the responses may well have been greater had still earlier growth stages been investi-

gated. Likewise, it may be presumed that size of inoculum and physiological age of the inoculum were important factors. Attention to such factors was limited to keeping them constant in any given set of experiments. Difficulties in reproducing the same degree of toxicity with a given concentration of an element were probably partly due to such factors.

The iron, molybdenum, and copper deficiencies noted during early but not during final growth stages are also of interest here. A similar observation has recently been made by Wooley (1941) with respect to the manganese requirement of *Lactobacillus casei*. The situation seems to be analogous to the effect on various microorganisms of certain organic growth factors whose addition may stimulate during early growth stages but be without effect on the maximum yield. Whether such results are due to a decreased lag period or an increased logarithmic growth rate remains to be seen.

Although the response of copper toxicity to iodine additions was clear-cut, the magnitude of the response, as well as the toxicities of given levels of copper, was variable, especially for lower copper concentrations. This was probably due to rapid removal of copper from solution by adsorption and precipitation, as well as to the factors mentioned above.

The manner of interaction of copper and iodine is not known. It may be pointed out that cupric ions and iodide ions react to form relatively insoluble cuprous iodide and free iodine.

Not all of the responses to iodides observed in these experiments are explainable on the basis of copper-iodide interaction. For example, in one experiment a significant response to 0.0001 ppm. of added iodine was noted. This is approaching the maximum dilutions at which the most active organic growth factors give perceptible effects. It has not been possible to demonstrate the copper-iodide interaction with either extremely low copper or iodine concentrations. It is likely, in view of the number of relatively insoluble iodides, that interactions exist between other metals and iodine. That this is true for silver ions and iodides has been noted by Stein-

berg (1935) in studying the growth of *Aspergillus niger*.

Preliminary experiments with flax and corn failed to demonstrate the copper-iodide interaction. The rapid precipitation of copper in our solutions, sometimes essentially complete within twenty-four hours, was probably the main reason for this result. The relatively greater sensitivity of these plants to iodine and lesser sensitivity to copper were probably also important. Nevertheless, it is likely that this type of action of iodine has entered into various plant nutrition studies with trace elements. Thus the experiments of Meyer (1931) with buckwheat show a toxicity in the presence of 0.125 ppm. of copper which is overcome by the further addition of 0.25 ppm. of iodine. The occasional occurrence of toxic substances in distilled water has been a question of some concern

to plant physiologists; in this connection, inhibition of such toxic actions should receive equal consideration.

SUMMARY

An interaction between copper and iodine in the early growth stages of *Azotobacter agile* has been demonstrated. The action of copper appears to manifest itself in a lengthened lag growth phase, while this effect is reduced by iodine. Such actions should be considered before stimulating effects of iodine are interpreted as evidence of the essential nature of iodine in plant or microbiological nutrition.

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SURFACE RELATIONSHIPS OF ROOTS AND COLLOIDAL CLAY IN PLANT NUTRITION ¹

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THE RECENT increase in national concern in regard to the losses of soil by erosion may well lead us to appreciate the fact that the land has gone nude because cover growth is prohibited by man's management and because of the declining store of fertility in the soil. This fertility consists of the chemical elements which the soil contributes to the plants. This contribution to any single crop is small. For many plants it represents but five per cent of the total dry matter in the plants, or even less. Air and water worked up into chemical combination by the energy of the sun constitute the bulk of most plants. This process of carbohydrate synthesis—the dominant one of all plant growth activities—can be carried out, or even initiated, only as the soil contributes from its store of essential plant nutrients. The declining supply of soil fertility must of necessity shift the plant population more and more to those kinds whose final composition represents less from the soil and more from the air and water. That is, considering

rather broadly the functions of the nutrient elements within the plant, the crops must shift toward those with less of protein and mineral content and more of materials with mere fuel significance. Naturally, lessened possibilities for proper animal and human nutrition must accompany these changes. In the light of these considerations, the contributions by the soil to plant contents, the mechanisms through which such contributions are made, and the relative supplies within the soil become of much concern to all of us.

MORE RECENT CONCEPTS OF MECHANISMS OF NUTRIENT DELIVERY BY THE SOIL.—With the increased knowledge of the colloidal behavior of the clay separate of the soil (Bradfield, 1923) and of the exchange of adsorbed ions between colloids through their contact (Jenny and Overstreet, 1938), it is no longer necessary to consider the supply of nutrients in the soil as limited to those in the displaceable soil solution. The immediate stock is not only that which is in true solution or that which would leach out, but also that which is exchangeable by other ions, more particularly those of similar electric charge. This exchangeable stock is of far larger magnitude than that of the simple solution. For better understanding

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of plant nutrition we must understand this cationic exchange behavior in which the soil gives up its adsorbed nutrient elements of positive charge for hydrogen, which is a cation contributed in exchange by the plant. Whether anionic behavior is similar is a question that is awaiting specific information, though it is not unreasonable to anticipate some likeness.

PLANT NUTRITION MAY BE MAINLY A SURFACE PHENOMENON.—If the cations of nutritional value are given up by the soil through this exchange in which the hydrogen, coming from the plant's liberation of carbon dioxide, displaces them from the clay in direct contact with the root, then the extent of the performance resolves itself into one of surface nature and areas. The rate of reaction, as well as the total of cations taken from a given soil by the plant roots, is then a question of magnitudes of root and clay surfaces in contact, and of the kinds and concentrations of adsorbed ions on the clay.

Should all other factors be removed from consideration, it will be of theoretical interest, at least, to view plant nutrition as largely a surface phenomenon determined by the surfaces of the plant root, and the surfaces of the soil or colloidal clay on which the exchange activities are possible. The following discussion uses some recent root surface data and some clay surface values in an attempt to elucidate plant nutrition hypothetically as an exchange phenomenon.

SOME ROOT SURFACE VALUES FOR PLANTS.—The studies by Dittmer (1940) give values for the root and root hair surfaces for soybeans, oats, rye and bluegrass in a specific soil. Since, in the last three of this group, more than 90 per cent of the surface of the root system is that of the root hairs, the values for the entire root system will be used, even though adsorptive activities are commonly attributed to the root hairs only. The values for the total surface of the roots are given in table 1 with figures ranging from 1.0 to 25.6 square centimeters per cubic centimeter of soil.

TABLE 1. *Total root surfaces of different crops in given soil volumes.*

Crops	Square inches per		
	42 cu. in. of soil	Cu in. of soil ^a	Sq. cm. per cc. of soil ^a
Soybeans	106.1	2.5	1.0
Oats	583.4	13.9	5.9
Rye (winter)	1,267.9	30.0	11.8
Bluegrass (Kentucky) .	2,779.9	66.1	25.6

^a Values are calculations from those by Dittmer.

SOME SURFACE VALUES FOR COLLOIDAL CLAY.—As for the surface offered by the soil for contact with the roots, this can be determined from the size of the particles of the colloidal clay. Numerous studies of this fraction of the Putnam silt loam subsoil have been made (Marshall, 1936). If we disregard the sand and silt fractions of this well weathered soil for their exchange activities, and if we accept the

general fact that this clay constitutes one-sixth of the surface soil of the Putnam profile, then the clay content will amount to .222 gm. per cubic centimeter of soil which weighs 1.33 gms. By placing the approximate general size of the clay particle at one-tenth micron, or .00001 cm. (10^{-5} cm.), as the effective diameter, the value will be that into which about 35 per cent of the clay falls. The shape of the particles is of disc nature (Marshall, 1941), but for the purpose of simpler concept, we may visualize the shape as cubical with faces of the above effective diametric dimensions, *viz.*, 10^{-5} cm.

SMALL PORTION OF SOIL'S CLAY CONTENT IS IN ROOT CONTACT.—Should we visualize that these colloidal clay cubes are carrying their adsorbed nutrients on their surfaces and are in contact with the root with one face of the cube against it, then each particle would present a contact area of 10^{-10} sq. cm. According to the root areas given in table 1, the numbers of clay particles which would be required to cover the entire root area in a cubic centimeter of soil are those given in table 2 (column 1). With a specific gravity of 2.5 and a particle volume of (10^{-5} cm.)³, the weight of the clay in root contact for the root surface per cubic centimeter of soil volume would be that given in the same table (column 2). Since there is but .222 gm. clay in this soil volume, then the clay in contact with the roots represents but a small percentage of the soil's total clay, or those fractions of per cent given also in table 2 (column 3).

ADSORBED NUTRIENTS REPRESENTED BY CONTACT EXCHANGE.—As a means of determining the amount of nutrients delivered by the clay surface in root contact, we may use the total exchange capacity of the clay, *viz.*, .65 M.E. per gm., or .65 pound equivalents per thousand pounds of clay, as has been reported from many determinations (Ferguson and Albrecht, 1941). In a cubic foot of soil weighing 83.2 pounds (1.33, volume weight, \times 62.4 pounds per cubic foot of water), of which one-sixth, 13.85 pounds, is clay, the exchange equivalents would be but .009 pounds ($13.85 \times .65/1,000$). When considered per acre six inches deep, this would be but 196.02 pound equivalents ($.009/2 \times 43,560$ sq. ft. acre area). Should this clay be saturated completely with calcium, then the clay per acre six inches deep would contain twenty times the equivalent, or 3,920 pounds of exchangeable calcium. If the percentage of surface in contact as given in table 2 is applied to this calcium value and if the calcium on the clay surface in contact is completely taken by the plants, then the amount of calcium so obtained by the different crops would be those in table 3 (column 1).

That removal to completion, or 100 per cent, by exchange is not easily conceivable as common occurrence was shown in the work by Ferguson and Albrecht (1941), where only 85 per cent of the exchangeable calcium was taken by three successive crops, but not without significant irregularities in plant growth. A single crop took but 40 per cent. If we should assume as usable only 85 per cent of the adsorbed calcium on the clay faces in root contact,

TABLE 2. *Clay in contact with plant roots expressed as numbers and weights of clay particles and per cent of clay in the soil.*

Crops	Clay in root contact per cubic centimeter of soil expressed as		
	Number of clay particles ^a	Weight, ^b gms.	Per cent of total clay ^c
Soybeans	1.0×10^{10}	$.25 \times 10^{-4}$.011
Oats	5.9×10^{10}	1.47×10^{-4}	.066
Rye (winter)	11.8×10^{10}	2.95×10^{-4}	.133
Bluegrass (Kentucky)	25.6×10^{10}	6.40×10^{-4}	.289
	(Column 1)	(Column 2)	(Column 3)

^a Root area in sq. cm. divided by area of face of particles (10^{-10} sq. cm.).^b Number of clay particles $\times (10^{-5})^3 \times 2.5$.^c Weight of clay particles $\times 100/.222$.

the amounts taken per acre would then be those in table 3 (column 2). If only a single crop had been grown with 40 per cent of the exchangeable calcium taken, then only those amounts as given in the table (column 3) would be taken per acre six inches deep.

NORMAL CALCIUM USE BY CROPS IS GREATER THAN THE DELIVERY BY DIRECT CONTACT ONLY.—When one considers the acre yields of these crops and the calcium contents of their above-ground parts as found by chemical analyses, then the fact is immediately evident that the soil delivers to this crop portion and particularly to the entire plants, roots and tops, more calcium than would be provided if only that adsorbed on the clay surface in immediate root contact were taken. In table 3 (column 4) are given the amounts of calcium commonly found in these crops as harvested from conservative acre yields.

MOVEMENT BETWEEN CLAY PARTICLES BY ADSORBED IONS IS SUGGESTED.—If these figures represent the facts, they suggest that there must be adjustments in concentration of the adsorbed ions, particularly nutrient ions, even on the colloidal clay surfaces of the individual particles and between the different clay particles. Calcium removal, according to these calculations for the commonly harvested parts of the crop, suggests adsorbed ion movement toward the roots through more than a few layers of clay particles, especially for the soybeans. When the entire plants are considered, the evidence is more convincing. Still further, the movement of ions cannot be wholly from clay in a silt loam of which only one-sixth is clay. Some of the root area is in contact with silt. From the mineral faces of the silt it would seem that the source would be the face in contact only. With the silt so little active, the clay

must be all the more active in the movement of adsorbed ions over the particle, and through several layers of particles.

MOVEMENT OF CATIONS BETWEEN CLAY PARTICLES DEMONSTRATED.—That such movements of ions from one clay area to another are possible has been demonstrated. By bringing a sand-clay mixture, the clay of which was saturated by calcium, into contact with a similar one saturated by hydrogen, the migration of these cations from one location to the other within a period of thirty days was demonstrated. Calcium had moved more than two inches into the hydrogen clay area, and the hydrogen had similarly moved into the calcium clay area. These migrations took place when all the calcium was adsorbed, and when no significant solution activity can be considered as playing a rôle in this exchange.

Cationic movement of adsorbed nutrients is a possibility, then, along the faces of the clay particles, if such activity may be visualized as occurring in the exchange atmosphere or in the adsorption layer on the face of the clay crystal, and if these plant and soil behaviors may be considered as evidence.

MOVEMENT OF IONS FROM MINERAL CRYSTAL INTO COLLOIDAL ADSORPTION ATMOSPHERE.—Since three successive crops may reduce the supply of exchangeable nutrients of the clay to 85 per cent of exhaustion, we are immediately confronted with the fact that continuous cropping on many experimental fields has gone forward for more than half a century without even approaching such a high degree of depletion of the exchangeable nutrients in the soil. How then is the supply on the exchange atmosphere of the clay maintained? Graham (1940), in his use of the colloidal hydrogen-clay in contact with pure minerals

TABLE 3. *Calcium available by root contact (pounds per acre six inches deep) and contained in normal crops.*

Crops	With contact exhaustion of surface calcium at			Content of normal crops Pounds
	100%	85%	50%	
Soybeans43	.36	.17	5.4 per ton forage
Oats	2.58	2.19	1.03	4.1 per 25 bu. grain and 1,200 lbs. straw
Rye (winter)	5.21	4.43	2.08	2.8 per 15 bu. grain and 1,000 lbs. straw
Bluegrass (Kentucky)	11.33	9.62	4.53	10.0 per ton forage
	(Column 1)	(Column 2)	(Column 3)	(Column 4)

of silt size, has demonstrated that the adsorbed hydrogen on the clay is active in exchanging itself for the cations of the mineral. This exchange serves to nourish plants (Graham, 1941). The silt fraction with its mineral store is then the supply from which that of the clay is replenished after depletion by plant growth.

ION MOVEMENT FROM SILT PARTICLES DIRECTLY TO PLANT IS POSSIBLE BUT SMALL.—That ions can be taken in some measure by the plants directly from mineral particles of silt size without the intervention of the clay has also been demonstrated by Graham (1941). Plants failed, however, to grow as well under such conditions as on the silt in the presence of other colloids less active than clay, and decidedly not as well as on the silt mixed with the colloidal clay. It was only when an acid clay was mixed with the minerals of silt size that the growth of the plants was most effective. Plants can then use the minerals directly and the silt size separates may serve, but their contribution is small. This may be a case of limitation strictly to surface contact area, since ionic movement from crystal to crystal does not seem so probable.

EXCHANGE CONCEPT CLARIFIES RELATION OF SOIL DEVELOPMENT TO CROP PRODUCTION.—Only in those soils, in which the mineral reserve is ample both as to the kinds and the amounts of necessary elements, will production be maintained for more than a three-year period of continuous cropping. Mineralogical studies of the silt fraction of the soil with its classification as dominantly quartz or "other-than-quartz" will contribute much to better understanding of the continued productivity of some of our lands. By knowing the extent to which the clay is exhausted of its nutrient cations, or the reciprocal, namely, the extent to which the clay has become saturated with hydrogen, and by knowing, in addition, the extent to which the reserve of mineral crystal nutrients in the silt fraction is exhausted, we can make some estimate of the degree of soil development and of the possibilities of crop production as to kind and quality. Such understanding of the soil in its practical significance is more easily obtained by aid of the concept of exchange between the colloidal clay and the root surface.

In the inorganic portion of the soil, then, the immediate supply of nutrients for plants would seem to

be on the colloidal clay in the adsorbed form. The "other-than-quartz" minerals of silt size would then seem to be the reserve supply for either direct or indirect use in the future, if not for part of the immediate growing season. The colloidal clay, then, aids through two steps in nourishing plants. In the first, through its root contact, it serves to deliver nutrients by cation exchange. In the second, it serves in connection with the mineral breakdown of the silt fraction of the soil. By means of this view of the soil and root behaviors mainly as contact and surface phenomena, we may visualize more clearly and interpret more simply the processes of plant nutrition, of depletion of soil fertility and others connected with crop production and soil maintenance.

SUMMARY

By means of some data giving the root surface per unit volume of soil and some giving the surface areas of colloidal clay, the calcium delivery to the crop through exchange phenomena was calculated. The calculations suggested that a crop gets more calcium than is present on only that clay surface in immediate root contact. The data suggested that exchangeable ions move from one clay particle to the next clay particle through several such layers.

Hydrogen movement from hydrogen clay into calcium clay and the reverse movement of calcium were demonstrated. Hydrogen clay contact with mineral crystals demonstrated similar exchange from the crystal to the clay colloid. Ionic movement from the mineral crystal to the root by direct contact failed to nourish the plants amply, yet the crystal in contact with the clay served effectively.

Thus in terms of these surface phenomena of the colloidal clay and the root, we may get a clearer concept of how the adsorbed nutrient supply of the clay is replenished from the mineral crystals of the soil. By means of this concept we can visualize more clearly the mechanism involved in plant nutrition, soil fertility depletion, and various aspects of crop production and soil maintenance.

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THE INFLUENCE OF VOLCANIC ERUPTIONS UPON POST-PLEISTOCENE FOREST SUCCESSION IN CENTRAL OREGON¹

Henry P. Hansen

THE DISTRIBUTION and abundance of peat bogs in North America is largely determined by the boundaries of Pleistocene glaciation. The occurrence of innumerable lakes within the glaciated region is responsible for their great number, and sufficient precipitation over much of this region permits the maintenance of a constant lake level or water table in the bog. Apparently most of the environmental conditions have been at an optimum for hydrarch plant succession culminating in the formation of peat deposits. Climate is perhaps one of the chief ecologic factors concerned with the development and continued accumulation of peat and other types of pollen-bearing sediments. The proximity of the Pacific Ocean and the presence of the Coast Range, Olympic Mountains, and the Cascade Range, permit the existence of a great diversity of climates, and hence, many plant formations in the Pacific Northwest. All of Oregon, and most of Washington and Idaho lie south of the boundaries of Pleistocene continental glaciation, and, consequently, peat deposits and other types of sediments suitable for pollen analysis are not common over much of this vast region. Where the climate is not too dry, the absence of ponded water has inhibited the initiation of hydrarch plant succession and subsequent accumulation of peat. The greatest number of peat deposits is in the Puget Lowland of western Washington, which was glaciated and where the climate is favorable for maximum bog development. Peat deposition has also occurred in montane glacial lakes and along the Oregon Coast, where the erosion cycle of a submerged shoreline has resulted in the formation of many lakes (Hansen, 1941c). In the latter region, sand dune lakes also support various stages of hydrarch plant succession, but they are usually recent or of uncertain age. East of the Cascade Range in Oregon and Washington the few existent lakes are usually too alkaline to permit hydrarch succession, or fluctuating water levels do not encourage peat deposition. In other areas the mature topography with its efficient drainage prevents the existence of standing water and subsequent hydrarch plant succession. Tule swamps are rare, and those examined overlie shallow strata of black muck, unsuitable for pollen analysis.

LOCATION AND CHARACTERISTICS OF THE BOG.—The peat deposit of this study is located about 13 miles west of Bend, Oregon, in section 16 of T. 18 S., R. 10 E. on the Three Sisters quadrangle. The elevation of the bog is about 5,240 feet above sea level. The peat and other pollen-bearing sediments have accumulated in Tumalo Lake, a small lake that was apparently ponded by the terminal moraine of

a valley glacier in a tributary of Tumalo Creek. The latter empties into the Deschutes River a few miles north of Bend. The Pleistocene mountain glaciers in this region evidently reached lower elevations, but post-Pleistocene volcanic activity has largely obliterated the surface effects of glaciation. In recent years the lake level has been raised by an artificial dam on the moraine at the lower end of the lake. In the autumn of 1940, the lake was drained to remove peat for commercial purposes, and a complete profile was obtained where the surface had not been disturbed. The hydrarch plant succession had reached a sedge-meadow stage with an abundance of *Hypnum* moss before the lake level was raised artificially. This stage of plant succession is similar to that of a montane bog on the east slope of the Cascades in central Washington, where the climate is also the same (Hansen, 1939c). The area of the peat deposit is about 300 feet wide and 600 feet long. The adjacent valley slopes rise directly from the edge of the bog. Peat samples were obtained with a Swedish-type peat borer. The depth of the sediments in the area of sampling is 7 meters, and test borings made across the bog show that the bottom is flat most of its width. This suggests that the lake was ponded in a U-shaped valley of glacial origin.

Two layers of pumice are present in the peat profile, one at 4.5 and the other at 2.0 meters. The occurrence of the pumice strata is significant, because the change in the edaphic conditions due to the deposition of the pumice in this region evidently had a marked influence upon the forest succession as suggested by the pollen profiles. There are several possible sources of the pumice, there having been considerable volcanic activity in Cascade Range of Oregon during post-Pleistocene time. It is probable that the pumice had its origin from one of the following: Mount Mazama, about 80 miles to the south; Newberry Crater, about 25 miles to the southeast; and Devils Hill,² about 11 miles to the west of the peat deposit (Williams, 1935, 1941). The eruption of Mount Mazama, forming the caldera holding Crater Lake, occurred during the post-Pleistocene between 5,000 and 10,000 years ago (Williams, 1941). The pumice mantle from this eruption extends with diminishing thickness to the east and for a distance of about 100 miles to the north. The site of the sediments of this study is located within a zone where the pumice is from 1 foot to 6 inches thick. It seems probable that at least one of the pumice strata had its origin from Mount Mazama. Two peat deposits farther south in the vicinity of Crater Lake, that lie definitely upon Crater Lake pumice, are 2 meters deep as determined by the writer. Assuming that the rate of deposition has been about the same in both

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² Personal communication from Dr. Howell Williams, University of California.

TABLE 1. Percentages of fossil pollen.

Depth in meters:	6.75	6.5	6.25	6.0	5.75	5.5	5.25	5.0	4.75	4.5 ^a	4.25	4.0	3.75	3.5	3.25	3.0	2.75	2.5	2.25	2.0 ^a	1.75	1.5	1.25	1.0	0.75	0.50	0.25	0.0
<i>Pinus contorta</i>	30	33	30	47	42	49	32	20	20	19	60	58	53	42	38	36	35	40	38	37	44	48	51	58	60	50	48	50
<i>P. monticola</i>	14	11	20	17	14	24	21	20	26	24	21	18	18	24	21	24	16	20	18	22	20	20	16	16	14	15	15	16
<i>P. ponderosa</i>	14	18	24	16	21	22	40	42	43	44	12	16	20	28	30	26	30	28	32	31	26	21	19	18	20	22	24	21
<i>P. lambertiana</i>	4	1	10	..	1	8	4	2	1	1	6	4	1	2
<i>Pseudotsuga taxifolia</i>	2	1	1	1	1	1	1	1	..	1	2	1	1	1	1	1	1	..	1	1	1	1
<i>Tsuga heterophylla</i>	1	1	..	1	1
<i>T. mertensiana</i>	3	2	3	3	2	1	2	2	1	4	4	2	2	2	6	4	3	3	4	3	3	4	3	6	4	6
<i>Picea engelmanni</i>	2	2	2	1	1	1	2	1	1	..	1	..	2	2	1	..	1	1	3	3	2	3	1	1	1	2
<i>Larix occidentalis</i>	33	28	10	12	2	1	..	4	1	1	1	1
<i>Abies grandis</i>	1	1	..	2	1	1	1	1	2	1	1	1	2	2	1	1
<i>A. concolor</i>	1	2	1	1	4	1	1	2	2	..	1	..	2	2	3	1	1	2	1	2	1	2	1	3	1	1	3	2
<i>A. lasiocarpa</i>	1	..	1	1	1	1	..	2	1	1	..	1	1	1	1	1	1	1
<i>Pinus spp.</i> ^b	17	22	17	14	19	26	25	25	12	11	9	15	12	21	15	17	15	18	13	12	10	18	16	18	19	17	15	12
<i>Abies spp.</i> ^b	1	3	2	1	1	1	3	5	1	3	1	1	2	1	1	1	3	4	5	1	1	1	1	1	2	4	3	2
Gramineae ^c	2	1	..	1	2	2	..	1
Compositae ^c	1	1	..	1	1	1	1	1	1	1	1	1
Chenopodiaceae ^c	1	..	1	1	2	1	..	1	1	1	1	1
<i>Alnus</i> ^c	4	4	2	2	1	..	3	1	2	..	2	1	1	4	..	1	1	1	2	2	..	1
<i>Betula</i> ^c	3	2	..	1	1	1	..	2	2	1	2	1	1	2	1	1	2
<i>Acer</i> ^c	6	5	1	2	2	..	2	1	2	2	1	1	1	2	1	1	1	1	1
<i>Salix</i> ^c	1	2	..	1	2	1	1	2	..	1
Ericaceae ^c	1	1
Cyperaceae ^c	1	5	2	8	2	3	6	7	3	7	3	2	3	3	3	4	7	7	6	11	3	7	13	20	2	6	7	9
<i>Nymphaeanthus</i> ^c	2	1	..	1	1	2	1	1

^a Levels at which pumice occurs^b Number of *Abies* and *Pinus* pollen grains discarded, not computed in the percentages.^c Number of pollen grains, not computed in the percentages.

areas, it suggests that the pumice at 2 meters came from the Mount Mazama eruption. If 6,000 years have elapsed since its eruption, the rate of peat deposition has been consistent with that of other post-glacial bogs in the Pacific Northwest (Hansen, 1942a). Thus, the lower pumice stratum must have come from a much earlier eruption of Mount Mazama or some other volcano. The separation of the two layers by 2.5 meters of fine peat indicates that the first eruption may have occurred about 12,000 years ago. Post-Pleistocene peat profiles in the lower Willamette Valley of western Oregon contain a single layer of pumice, less than 2 meters from the present surface of the sediments (Hansen, 1942b). It is possible that this layer owes its origin to the same volcanic eruption as the upper stratum in the peat profile of this study.

In the preparation of the peat for microscopic study, the potassium hydrate method was used. From 150 to 200 pollen grains of significant species were identified from each level, except the lowest at 7 meters, where no pollen was present. The size-range method, which has been described in several recent papers, was used in separating the species of *Pinus* and *Abies* (Hansen, 1941a, 1941b, 1941c). In this study it should be noted that pollen listed as that of *Pinus monticola* probably includes some of *P. albicaulis* which is present near timberline. Also pollen listed as that of *P. ponderosa* may include small proportions of *P. albicaulis* and *P. lambertiana* due to a slight overlap of their size-ranges. In the firs, pollen identified as *Abies nobilis* may include some of *A. concolor*, although the small proportions of fir pollen present make this source of error negligible. The size-range method in the identification of fossil winged conifer pollen is shown to be feasible by the consistency of pollen profiles in certain peat profiles. In four peat profiles from Lower Klamath Lake of Oregon and California, the pollen profiles of each species of pine are similar in their major and significant trends (Hansen, 1942a). The separation of western yellow and western white pine is important because of the climatic indicator value of these species. The identification of lodgepole pine pollen is also essential, not for the interpretation of climatic trends, but rather for the recorded interruption of forest succession due to changes in edaphic conditions or other non-climatic environmental factors. It is particularly significant in this study. Pollen is abundant at most levels, with more than 1,000 pollen grains present on a single slide at many horizons. This is perhaps due to the fine type of sediments, which require a long period of time for their deposition, resulting in a concentration of pollen of many seasons in a small unit of thickness. The enormous quantity of pollen shed by the various species of pine is also a factor in the abundance of pollen present. Those species recorded by less than 1.5 per cent are listed in the table as 1 per cent.

FORESTS IN ADJACENT AREAS.—The terrain of the region surrounding the bog is extremely rugged with considerable relief. The divide of the Cascade Range

is about 15 miles to the west. The South Sister, reaching an elevation of 10,354 feet, rises about 13 miles to the northwest and supports large glaciers. Broken Top peak, about 10 miles to the northwest, also has glaciers, and there are many peaks within several miles that attain a height of over 6,000 feet. The elevation decreases to the east, with the less rugged regions lying at an altitude from 3,000 to 4,000 feet. This great relief permits the existence of several life zones within a few miles of the site of the sediments. The bog lies within the timbered Arid Transition area (Bailey, 1936). The rapid increase in elevation to the west causes this to grade into the Canadian which in turn grades into the Hudsonian zone at still higher altitudes. The Arctic-alpine zone occupies considerable areas on the upper slopes of the Three Sisters. At lower elevations to the east the timbered Arid Transition gives way to the timberless part of the same area, which in turn grades into the Upper Sonoran zone still farther to the east. Much of the timbered Transition is forested with western yellow pine (*Pinus ponderosa*), which forms a zone of varying width along the eastern flank of the Cascade Range in Oregon and Washington. This zone, however, is broken and discontinuous for a distance of about 100 miles from Crater Lake to Bend. Vast forests of lodgepole pine (*Pinus contorta*) occupy the pumice-covered areas that extend north and east of Crater Lake. Forest type maps (1936) show an almost uninterrupted belt of yellow pine east of the lodgepole pine forests where the pumice mantle is not so thick as farther to the west. North of Bend, the lodgepole pine zone tapers to nothing, and is practically absent for the last 100 miles to the Columbia River. This suggests that the prevalence of lodgepole pine in this region is due to the pumice mantle, and that the other ecological conditions are such as normally to support a forest of yellow pine over most or all of the timbered Arid Transition.

At some points the lower limits of the yellow pine zone grade into sparse forests of western juniper (*Juniperus occidentalis*), which occupy a considerable area in central Oregon. In other places the yellow pine forest gives way directly to the bunchgrass prairie. Near the upper boundaries of the yellow pine zone, sugar pine (*P. lambertiana*), Douglas fir (*Pseudotsuga taxifolia*), lowland white fir (*Abies grandis*), white fir (*A. concolor*), western red cedar (*Thuja plicata*), incense cedar (*Libocedrus decurrens*), and western larch (*Larix occidentalis*) may be found on favorable sites. Sudworth (1908) gives the southern limits of the range of western larch as the headwaters of Squaw Creek, 10 miles to the northeast of Tumalo Lake. A few specimens, however, were noted in the vicinity of the lake.

The most common species of conifers in the Canadian zone immediately above the Transition are western white pine (*P. monticola*), western hemlock (*Tsuga heterophylla*), Engelmann spruce (*Picea engelmanni*), noble fir (*Abies nobilis*), silver fir (*A. amabilis*), Douglas fir, lowland white fir, and lodgepole pine. In this zone lodgepole pine also occupies

the pumice-covered areas, or areas where fire has been to its advantage. Next to lodgepole, western white pine is perhaps the most abundant species in this zone. The Hudsonian zone is more sparsely forested than the Canadian, and the principal species are mountain hemlock (*Tsuga mertensiana*), white bark pine (*P. albicaulis*), alpine fir (*A. lasiocarpa*), and Alaska cedar (*Chamaecyparis nootkatensis*). The boundaries of these zones are irregular, the forests of each extending upward or downward beyond the general limits where conditions are favorable.

The peat deposit is located within a climatic province designated by Thornthwaite (1931), as being subhumid and microthermal, with adequate precipitation at all seasons. The climatic zones are narrow in this region, because of the steep slope of the Cascades. The precipitation increases at higher elevations to the west and decreases at lower altitudes to the east. The mean annual precipitation at Bend, 13 miles to the east and at an elevation of 3,629 feet, is 13.21 inches, and at Sisters, about 20 miles to the north at an elevation of 3,175 feet, it is 16.65 inches (Climatic Summary, U. S. D. A.). At Bend, about 27 per cent occurs during May to September inclusive, and at Sisters, about 21 per cent falls during the same period.

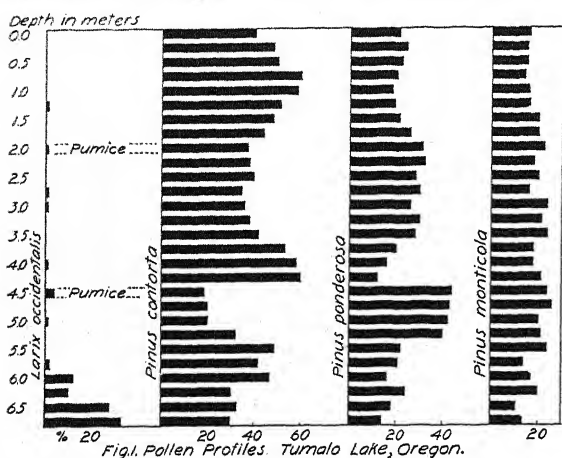
POST-PLEISTOCENE FOREST SUCCESSION. — The depth of the sediments suggests that they represent the greater part of postglacial time. The fineness of the sediments and the type of vegetation contributing to their deposition indicate that the rate of sedimentation was slow. The average depth of six post-Pleistocene peat deposits studied in eastern Washington and Oregon is 6 meters, which is slightly less than the 7-meter depth of the bog of this study. As previously stated, the eruption of Mount Mazama occurred not less than 5,000 nor more than 10,000 years ago. If the upper layer of pumice at 2 meters owes its origin to this eruption, then the age of the peat profile is perhaps 15,000 years. If the lower stratum at 4.5 meters represents this eruption, then the sediments had their origin somewhat later. The total depth of the sediments, however, suggests the former figure.

The forests recorded in the lowest pollen-bearing level at 6.75 meters consisted chiefly of western larch, lodgepole pine, western white pine, and western yellow pine. The first was apparently the most abundant, and contributed 33 per cent of the pollen (fig. 1). Lodgepole, western white, and western yellow pine are represented by 30, 14, and 14 per cent, respectively. It is probable that larch was predominant by a greater margin than is denoted by the pollen proportions, because this species does not shed nearly so much pollen as the pines, especially lodgepole. Larch pollen percentages decline rather abruptly from the lowest level upward, to show only 2 per cent at 5.75 meters. It is then recorded sporadically in low proportions to the surface. Larch is not recorded by its pollen to any great extent in Pacific Northwest bogs, even though they are located within

forests including appreciable proportions of this species (Hansen, 1939a, 1939c, 1940b, 1941e). The highest proportion present is 25 per cent in a bog near Spokane, Washington (Hansen, 1939b). In Wisconsin bogs, tamarack (*Larix laricina*) is only sparsely represented, even though it is the chief tree on bogs in their climax stage (Hansen, 1937, 1939d). Apparently the pollen of species of this genus is not well preserved in peat. The predominance of larch in the lower horizon is significant in that the bog lies near the southernmost limits of its geographic range in Oregon. The sporadic occurrence of its pollen in the upper part of the profile indicates that it was never abundant after its initial predominance. The proportions of larch pollen in the lower four levels suggests the occurrence of repeated fires prior to initial sedimentation. Larch has thick bark and is able to withstand successive severe fires that may destroy first the parent trees of other species, and then their seedlings. The lack of competition for light then permits it to flourish until other species are able to regain a foothold. If undisturbed by fire, these will eventually replace the larch (Sudworth, 1908; Larsen, 1929). Lodgepole pine may also increase in abundance after fire, but this is due to release of seed from the cones as a result of heat, rather than survival of the parent trees. Subsequent fire before seed-bearing age may completely eradicate this species.

The several species of pine show a general increase to 5.5 meters, with lodgepole pine becoming predominant (fig. 1). Yellow pine is then recorded as increasing abruptly to 44 per cent at 4.5 meters, white pine remains constant, and lodgepole pine decreases to 19 per cent. Most of this region is normally forested with a yellow pine climax, and it seems probable that the initial increase in this species depicts a warming and drying of the climate as the effects of recent glaciation were modified. Modification of the conditions left by the hypothetical fires may also have been a factor in its increase. Lodgepole pine shows a sharp increase from 19 per cent at 4.5 meters, whereas yellow pine declines to only 12 per cent at this level. White pine remains more or less constant. It is extremely significant that a stratum of pumice is present at 4.5 meters, indicating that a volcanic eruption occurred at this time, depositing a pumice mantle over this region. This apparently resulted in an increase in lodgepole pine due to the change in the edaphic conditions or possibly because of further fire. The depth of the pumice was probably not sufficient to kill the existing yellow pine forests outright. The changes in the edaphic conditions, however, may have been unfavorable for yellow pine seedlings, and lodgepole was thus able to thrive because of the absence of competition. The general constant trend in the pollen proportions of white and white bark pine during this interval suggests that they were unaffected by the pumice because of their existence at higher elevations to the west, where the pumice mantle is thinner or absent. Lodgepole pine is recorded as generally declining

from 1.25 to 2 meters, yellow pine shows a slight increase, and white and white bark pine continue their static trend. The increase in yellow pine suggests a partial return of this species as the pumiceous soil perhaps was gradually modified by climatic and biotic factors. Lodgepole is again indicated as increasing, less gradually than at first, upward from the 2-meter horizon. The occurrence of a second layer of pumice at this level records another volcanic eruption and deposition of volcanic material, with accompanying changes in edaphic conditions favorable



for lodgepole pine. Although it seems probable that the source of this pumice was the eruption of Mount Mazama, an increase in erosion may have washed the pumice into the lake from adjacent slopes. Erosion greater than normal may have resulted from deforestation by fire, which in itself would cause edaphic and biotic changes favorable for an increase in lodgepole pine. Lodgepole shows an increase upward to .75 meter, and then it declines slightly to the surface. Yellow pine is recorded as slightly declining from 2 meters for several levels upward, and then it remains constant to the surface. White pine also maintains its static trend to the top. The surface sediments record 50, 21, and 16 per cent for lodgepole, yellow, and white pine, respectively (fig. 1). It seems probable that lodgepole is over-represented because of its extensive existence windward to the site of the sediments. Conversely, yellow pine is under-represented because of its existence largely leeward to the bog.

Other coniferous species recorded by their pollen, either consistently or sporadically throughout the peat profile, are sugar pine, western and mountain hemlock, Douglas fir, Engelmann spruce, and lowland white, alpine, and noble fir. Mountain hemlock and noble fir are most abundantly and consistently represented. Sugar pine pollen is present in the lower levels, but is entirely absent in the upper 2 meters. Grass, Composite, and Chenopod pollen occurs sporadically in the profile, but these species were never extensive near the site of the sediments. Broadleaf trees including alder, birch, maple, and willow are sparsely represented. Sedge pollen is present at all horizons and shows a slight increase

in the upper levels as the sedge-meadow stage of hydrarch succession progressed (table).

CLIMATIC CONSIDERATIONS.—There seems to be little evidence for climatic trends in the pollen profiles of this study. The increase in yellow pine from the bottom to 1.5 meters may mark a drying and warming during the early postglacial, but its continued development was apparently interrupted by the deposition of the pumice mantle. Desiccation of postglacial climate is denoted by pollen profiles of other peat deposits east of the Cascades in Washington and Oregon. Sediments from Lower Klamath Lake record drying and warming by an increase in yellow pine to a maximum from the bottom to about halfway up in the profiles (Hansen, 1942a). This was followed by slightly cooler and moister conditions. A bog near Spokane, Washington, located in a yellow pine climax, records a sharp increase in grasses, Composites, and Chenopods, marking a hot, dry period during the middle third of the post-Pleistocene (Hansen, 1939b). In north central Washington, pollen profiles suggest a gradual drying to a maximum that has persisted to the present (Hansen, 1940b). In the lower Willamette Valley of western Oregon, three peat profiles record an influx of white oak (*Quercus garryana*) of considerable magnitude in the upper levels (Hansen, 1942b). The pollen profiles of this species and a decline in Sitka spruce (*Picea sitchensis*) and lowland white fir from the bottom upward is significant evidence for a dry period during the second half of the post-Pleistocene in the Willamette Valley. Pollen profiles of a peat deposit in the eastern foothills of the Coast Range in west central Oregon also provide evidence for warming and drying during this period (Hansen, 1941b). In other parts of the Pacific Northwest west of the Cascades, precipitation has probably not been a limiting factor in postglacial forest succession (Hansen, 1938, 1940a, 1941a). In a 7-meter profile of lake sediments in the Upper Sonoran life zone of east central Washington, the relative succession of grassland and forest suggests a warming of the climate during the postglacial to a degree persisting to the present (Hansen, 1941d).

Another source of evidence for a dry period during the middle third or latter half of postglacial time is the occurrence of artifacts underlying 6 to 8 feet of fibrous peat in Lower Klamath Lake (Cressman, 1940). The lake was drained in 1917, and wind and fire removed the fibrous peat, exposing an extensive artifact horizon. This signifies that the lake dried up sufficiently to permit early man to build his camp sites upon the exposed bed. A later increase in moisture resulted in inundation of the lake bed and the subsequent deposition of 6 feet of tule peat. Antevs (1940) estimates this period of desiccation to have occurred between 7,500 and 4,000 years ago. This dry period may have been synchronous with that indicated by the pollen profiles mentioned above. The occurrence of a postglacial dry period is further substantiated by the salinity of certain lakes in the Great Basin. The present salinity of these lakes is

too low to represent a continuous deposition of salts during the entire post-Pleistocene (Antevs, 1938). Apparently the lakes dried up, and their precipitated salts were removed by wind or buried. The present lakes were reborn in the freshened basins about 4,000 years ago. This is believed to have been synchronous with the reinundation of the Lower Klamath fossil lake bed. Another line of evidence for a dry period is that the remnants of most Pleistocene glaciers in the western mountains entirely disappeared during the postglacial, and the present glaciers were formed only a few thousand years ago (Matthes, 1939). Their wasting and rebirth are thought to have resulted from decreased precipitation followed by an increase in moisture. Pollen profiles in eastern North America also indicate a period of maximum warmth and dryness, succeeded by an increase in moisture and some cooling in recent time. The author hopes to show that all or most of these lines of evidence for a dry postglacial period are chronologically correlated.

SUMMARY

A montane peat deposit on the east slope of the Cascade Range in central Oregon records post-Pleistocene forest succession that was apparently influenced more by the deposition of pumice than by climate. At least two volcanic eruptions are evidenced by the presence of pumice strata in the peat profile.

There are four major trends of forest succession as interpreted from the pollen profiles. An initial predominance of western larch suggests the occurrence of repeated fire just prior to the origin of the sediments. When the effects of the hypothetical fires had been modified, lodgepole pine assumed predominance for a short time, only to be superseded by western yellow pine as the climate became warmer and dryer. Yellow pine never reached a maximum, however, because its trend was interrupted by a pumice fall that caused unfavorable edaphic conditions for its continued development. Lodgepole pine once again became predominant.

Lodgepole retained its predominance during the rest of the post-Pleistocene, but yellow pine gained slowly until another pumice fall interrupted its climax trend. Lodgepole pine made a less precipitate gain, but it attained the same proportion as it did after the first eruption. It is believed that the yellow pine forests which lie chiefly leeward to the bog in this region are under-represented by their pollen in the profile. The source of the pumice is not known with certainty, but it seems probable that at least one, probably the upper layer, came from the eruption of Mount Mazama between 5,000 and 10,000 years ago.

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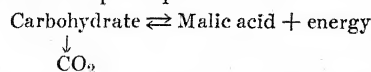
METABOLISM OF ORGANIC ACIDS IN EXCISED BARLEY ROOTS AS INFLUENCED BY TEMPERATURE, OXYGEN TENSION AND SALT CONCENTRATION ¹

Albert Ulrich

In a previous paper (Ulrich, 1941) data were reported on the relationship between cation and anion accumulation and organic acid metabolism in excised barley roots. The general conclusion was that when cations were absorbed in excess of anions from the external culture solution, the ionic balance in the root cells was adjusted by the formation of organic acids and that conversely, when anions were absorbed in excess of cations, the organic acid content of the roots decreased. In the present paper the effects of temperature, oxygen tension and salt concentration of the external culture solution upon the organic acid content of excised barley roots will be presented.

The technique employed for studying the metabolism of organic acids in excised barley roots has been reported (*loc. cit.*), and will be summarized only briefly. Under a carefully standardized procedure barley seeds were germinated and grown in darkness for five days and sixteen hours. At the end of this period, the excised barley roots were placed into the desired culture solution maintained at a definite temperature and constant oxygen tension (Ulrich, 1940). Following the removal of the roots after treatment in the culture solution, they were centrifuged to remove excess solution and stored at -18°C . until chemical analyses could be made on the expressed sap.

EFFECT OF TEMPERATURE ON ORGANIC ACID CONTENT OF EXCISED ROOTS.—The organic acid content of plants has generally been reported to be inversely related to changes in temperature (Bennet-Clark, 1933b; Wolf, 1931, 1938). Wolf (1931) considers that the rate of formation of organic acids is decreased at low temperatures, but that at the same time their rate of decomposition is decreased to an even greater extent. At higher temperatures the rate of decomposition is increased faster than the rate of formation and consequently the quantity of organic acids decreases. Bennet-Clark (1933b) believes the formation of organic acids has the following schematic relationship, and accordingly should follow the Le Chatelier principle.



This principle was apparently satisfied, when Bennet-Clark showed that the organic acids previously accumulated were soon lost from detached leaves of *Sedum praealtum* kept in darkness at 27°C . The low acid content was maintained until the leaves were

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transferred to a lower temperature (3°C .), when a gain in acids was recorded. Similar leaves kept initially at 3°C . showed a gain which was followed by a loss in acids after transferring to a temperature of 27°C .

In order to determine if excised barley roots behaved in a similar manner, sets of roots were placed for eight hours in 0.0005 M CaSO_4 solution maintained at 5° , 15° , 25° , or 35°C . (previously brought into equilibrium with air by aeration at the desired temperature). The results are recorded in figure 1.

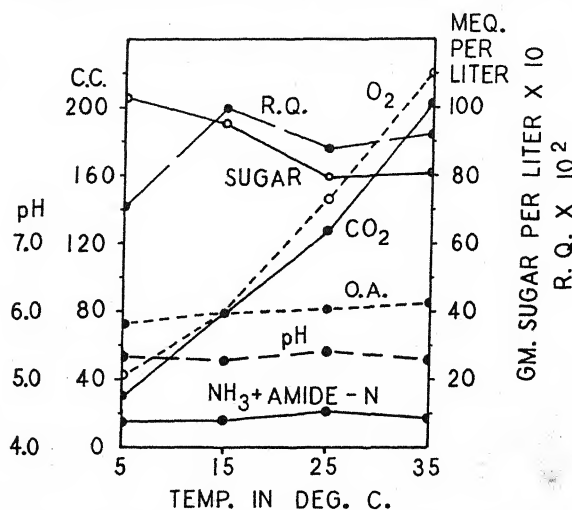


Fig. 1. Effects of temperature on the metabolic activity of excised barley roots in 0.0005 M CaSO_4 solution for eight hours. CO_2 and O_2 are given in cubic centimeters for 100 grams of excised roots. R.Q.— CO_2/O_2 . Sugar—total sugars expressed as grams glucose per liter of expressed sap. The following are given as milliequivalents per liter of sap: O.A.—total non-volatile organic acids; NH_3 + amide—N.—ammonia plus amide nitrogen. pH values were determined on expressed sap.

The total organic acid content of roots in the 0.0005 M CaSO_4 solution (36.5, 39.8, 40.7, 42.8 meq. per liter at 5° , 15° , 25° , and 35°C . respectively) changed only slightly even though the total respiration, as shown by the carbon dioxide evolved and by the oxygen absorbed, was approximately doubled for each 10°C . rise in temperature. With the exception of significant decreases in sugar content, other factors which might have been influenced by temperature showed little or no change. The changes in the hydrogen ion activity and in amide plus ammonia nitrogen concentrations were insignificant, although the latter tended to increase slightly from 15° to 35°C . Even though significant decreases in total sugar content occurred at all temperatures studied, there remained a large supply of sugar in all roots at the end

of the experiment, so that a deficiency of sugar could not have limited organic acid formation. The relatively large decrease in respiratory quotient at 5°C. is not to be considered significant, since the errors in setting the zero point in the mercury manometer are relatively great when measuring small quantities of oxygen. These errors do not occur in the carbon dioxide determinations, and these values may, therefore, be used for comparative purposes.

EFFECT OF TIME AND TEMPERATURE ON ORGANIC ACID FORMATION.—Using the same culture solution as in the previous study (0.0005 M CaSO_4), and the temperatures of 5°, 15°, and 25°C., the observation periods were extended from eight hours to a maximum of fifty-six hours (fig. 2-4). For each time interval and temperature a separate set of roots was taken. The results (including buffer curves not shown here) indicate that if there is a time element involved in the formation of organic acids at temperatures ranging from 15° to 25°C., it must be beyond 56 hours. At 5°C. there is a small increase in organic

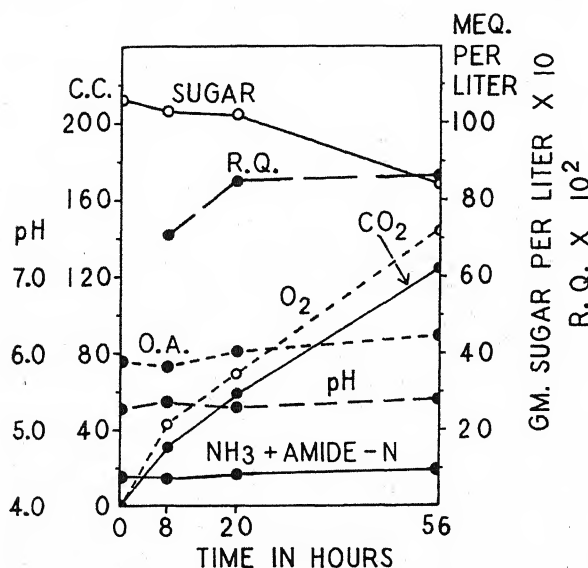


Fig. 2. Effects of time on the metabolic activity of excised barley roots in 0.0005 M CaSO_4 solution maintained at 5.0°C. (For explanation of values see figure 1.)

acids at the end of 56 hours, which, however, may not be significant. In the light of the data previously presented (Ulrich, 1941) the extent of this organic acid increase could be intimately related to the hydrogen ion deficit (pH increase) induced by metabolic changes at the lower temperature.

Other noteworthy points arising in the time-temperature study may be illustrated by the results of the experiments at 25°C. for the time intervals of 8, 20, 32, and 56 hours (fig. 4). The respiratory activity (fig. 5) decreased gradually for the first 32 hours and then increased slightly for the 44- and 56-hour periods. During the 56 hours (fig. 4) the total sugar content decreased from an average value of 10.6 grams to 1.20 grams per liter of sap, while

the organic acid content remained practically constant. If there were a dynamic equilibrium between total sugars and organic acids in the barley roots as postulated by Bennet-Clark (1933b) for succulents it would be expected that the organic acids might likewise be depleted. Under the present conditions a

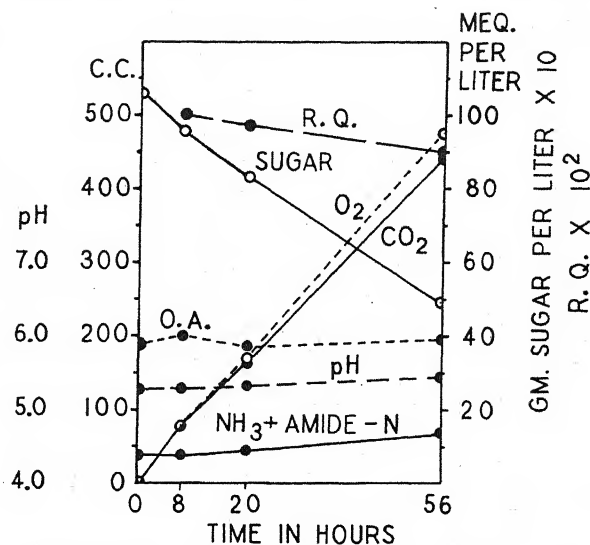


Fig. 3. Effects of time on the metabolic activity of excised barley roots in 0.0005 M CaSO_4 solution maintained at 15.0°C. (For explanation of values see figure 1.)

decrease in the organic acids failed to materialize except when the root cells had been depleted of total sugar for some time. Then the organic acids decreased; the inference was drawn that they were utilized in respiration (Ulrich, 1941). Furthermore, the required relationship of organic acids to the rate of CO_2 output (high CO_2 production, low organic

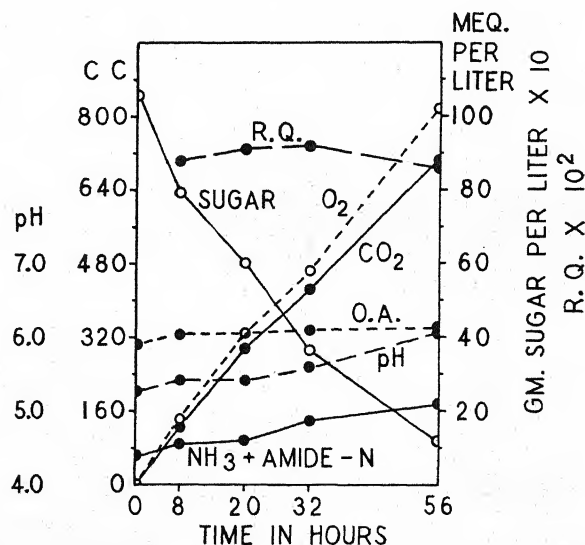


Fig. 4. Effects of time on the metabolic activity of excised barley roots in 0.0005 M CaSO_4 solution maintained at 25.0°C. (For explanation of values see figure 1.)

acids, and *vice versa*) as observed by Bennet-Clark in *Crassula lactea* was not apparent with barley roots, since under the present experimental conditions there were no ionic changes requiring the formation or loss of organic acids as would occur when roots absorb from salt solutions cations in excess of anions (e.g., K_2SO_4 , KH_2PO_4 , K citrate, $KHCO_3$) or anions in excess of cations ($CaBr_2$, and under certain conditions, KBr). The results of Thoday and Jones (1939) with *Kleinia articulata* likewise are not explained by Bennet-Clark's hypothesis, since they failed to find the required fluctuations in respiration.

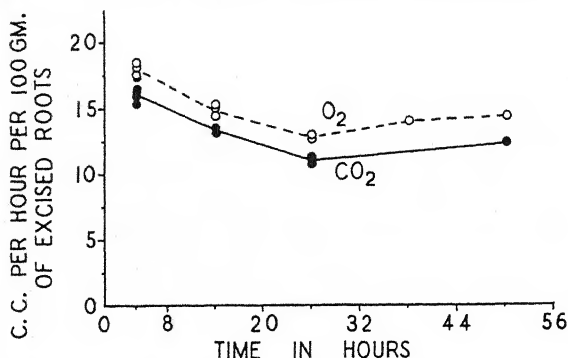


Fig. 5. Oxygen absorbed and CO_2 evolved by 100 gm. of excised barley roots in 0.0005 M $CaSO_4$ solution maintained at $25^\circ C$.

The increase in pH at the 32- and 56-hour intervals was most likely the result of an increase in ammonia in the ammonia plus amide fraction (fig. 4). This was not accompanied by a corresponding increase in organic acids as would be expected from Ruhland and Wetzel's (1926, 1927) theory, according to which the organic acids are thought to be formed from oxidative deamination of amino acids. The failure of organic acid formation is also not in agreement with the theory proposed here that the acids are produced from carbohydrates as a result of a tendency towards increase of pH within the root cells. However, this failure to form acids, according to the latter hypothesis, may be due to the very low sugar content of the cells at the time of the pH change. This contention is supported by evidence of the loss in organic acids in the 56-hour KBr treated roots in which the total sugars were at a minimum (Ulrich, 1941, fig. 5).

Another point of interest is the effect of potassium bromide on respiration. At all temperatures except $5^\circ C$. the respiration is significantly higher in a solution of KBr (*loc. cit.*, fig. 3) from which active salt absorption occurs than in a $CaSO_4$ solution (fig. 1) from which few ions are absorbed. If the total respiration is considered, this same relationship holds true for the time studies except in the 56-hour period. Here in the KBr set (*loc. cit.*, fig. 5) the sugar decreased more than in the roots in the calcium sulfate solution (fig. 4), so that for this time interval the latter roots respired in excess of those in the potassium bromide solution. The higher initial respira-

tory activity of potassium bromide treated roots may be associated with effects on metabolism through the absorption of certain potassium salts. Steward and Preston (1941) have shown in experiments on potato discs that increased protein metabolism accompanied increased respiration, but so far protein metabolism has not been found to be a conspicuous feature in the case of roots for the limited time periods of the experiments.

RELATION OF OXYGEN TENSION AND SALT SOLUTIONS TO ORGANIC ACID FORMATION.—The necessity for maintaining the proper oxygen tensions in actively absorbing tissues was recognized by Steward (1932) with potato discs, and more recently by Hoagland and Broyer (1936) with excised barley roots. The possibility that oxygen tension might influence the organic acid content of succulents was considered during Pfeffer's time to be related to the morphological structure of such plants. Gaseous interchange was thought to be difficult, and it was therefore, considered that succulents, particularly the more fleshy types, would conserve their CO_2 at night by forming organic acids; on the following day these would be decomposed by light to make CO_2 available for photosynthesis. In this connection Warburg (1886) and subsequently Astruc (1903) and others, found that increased oxygen tensions increased the organic acid content of succulents either in the light or dark, while the converse was true for low oxygen tensions. The work of Richards (1915) on *Opuntia versicolor* contradicted this general conclusion. Since all these workers used the direct titration method to determine organic acids, their results are open to question. More recently Bendrat (1929) found with *Sempervivum glaucum* that in the absence of oxygen (vaselined leaves) there was no change in total acids, although there was a decrease in titratable acidity (method used by Warburg). Wolf (1931) repeated this work by placing leaves in a vessel which was then exhausted and refilled with hydrogen several times. He ascertained from the total organic acid content that in some cases acidification during the night was inhibited completely, while in others the inhibition was partial, thus verifying Warburg's earlier work.

In the present investigation the circulating system adopted for the study of respiration in excised barley roots was readily adaptable to investigating the effect of various oxygen tensions upon root metabolism, and upon the respiratory quotient (Ulrich, 1940). After a given oxygen-nitrogen mixture had been introduced into the apparatus, the oxygen content was maintained automatically at a constant value by the oxygen generator. In this manner one of the major difficulties arising in oxygen tension studies with closed systems was entirely eliminated. The results from experiments with excised roots held for eight hours at $25^\circ C$. and using various oxygen tensions and salt solutions are given in table 1.

In the series of experiments with 0.0005 M $CaSO_4$ solution and different oxygen tensions (table 1) the effect of oxygen tensions on metabolism in certain

TABLE 1. *Influence of oxygen tension on organic acid formation.*^a

Culture solution	Treatments % O ₂	Total CO ₂ produced cc.	O ₂ ab- sorbed cc.	R. Q. CO ₂ /O ₂	Composition of expressed sap					
					Total sugars gm./L	pH	Total O. A. meq./L	Meq./L absorbed	NH ₃ -N + CONH ₂ -N meq./L	
Original roots ^b	10.6	5.27	37.9	7.8
0.0005M CaSO ₄	0.0	97.7	7.1	5.38	27.5	4.4
	pure N ₂									
0.0005M CaSO ₄	3.2	97.5	79.9	1.22	9.6	5.39	37.4	7.6
0.0005M CaSO ₄	Air	128.0	145.7	0.88	8.0	5.42	40.7	10.6
0.0005M CaSO ₄	87.9	150.3	162.0	0.93	8.5	5.28	39.1	8.8
0.005M KBr	4.7	104.6	98.0	1.07	8.7	5.44	45.6	22.1	19.0	7.3
0.0005M CaSO ₄										
0.005M KBr										
0.0005M CaSO ₄	3.8	111.5	80.8	1.38	9.5	5.49	43.7	22.9	18.8	6.2
0.005M KBr										
0.0005M CaSO ₄										
0.005M KBr	Air	173.5	186.2	0.93	7.5	5.44	45.1	33.7	26.8	9.3
0.0005M CaSO ₄										
0.005M KBr										
0.0005M CaSO ₄	85.0	177.5	190.2	0.93	7.1	5.33	47.2	31.5	23.0	7.5
0.005M KBr										
0.0005M CaSO ₄										
0.005M KNO ₃	3.5	119.8	95.3	1.26	8.3	5.65	48.6	28.2 ^d	16.2	8.1
0.0005M CaSO ₄										
0.005M KNO ₃										
0.0005M CaSO ₄	Air	186.0	200.9	0.93	5.9	5.69	52.8	50.8 ^d	24.1	10.2
0.005M KNO ₃										
0.0005M CaSO ₄										
0.005M KNO ₃	Air	197.1	202.7	0.97	6.2	5.66	61.5	52.8	32.6	11.1
0.0005M CaSO ₄										

^a One hundred gm. centrifuged roots in 3 liters of culture solution at 25°C. for eight hours.^b Average of 10 values. Standard deviation (s) for total O. A. equals 2.5 milliequivalents per liter (table 1, Ulrich, 1941).^c Average of 7 values. Standard deviation (s) for total O. A. equals 4.1 milliequivalents per liter (table 2, Ulrich, 1941).^d Determined from culture solution analyses assuming 100 per cent solubility of the potassium in the expressed sap. (Final root weight minus dry weight of press cake equals total expressed sap.)

aspects is noteworthy. The excised roots subjected to pure nitrogen (introduced into the circulating system by passing through pyrogallol; the oxygen generator was not in operation) showed the greatest change. The quantity of CO₂ evolved was still appreciable, and may have arisen in part from the residual oxygen not entirely removed from the roots at the start of the experiment, but probably in the main from anaerobic respiration. It is interesting to observe the large loss in total organic acids and sugar content. Since the roots held under an anaerobic condition during the eight-hour period were undoubtedly injured, part of these losses may have been caused by a direct loss of solutes, as indicated by turbidity of the final culture solution and by significant loss in root weight. While these changes were taking place, the combined ammonia and amide content was reduced slightly. This diminution may be associated with root injury or may be reconciled with the generally accepted viewpoint that oxygen is necessary for the formation of amides (Onslow, 1931; Chibnall, 1939), assuming here that the ammonia determined represented labile amides.

When excised roots were placed in 3.2 per cent oxygen, the reduction in respiration was significant. The low oxygen pressure resulted in an increase of the respiratory quotient, so that it was significantly greater than one. In spite of the low oxygen tension, the organic acid content was not lowered signifi-

cantly, indicating that extreme oxygen deficiencies must occur before appreciable amounts of these compounds are lost. The sugar loss was not as great as in pure nitrogen or in air. The ammonia + amide content was not greatly influenced, although the quantity of these constituents was lower than in the aerated roots.

An increase in oxygen content from 20 to 87.9 per cent did not alter the results significantly, but there was an increase in respiratory activity which might be attributable to the higher oxygen tension. There is no great oxygen deficiency in air, otherwise the respiratory quotient would be greater than one. The organic acid content, as before, was not influenced. Neither were the ammonia + amide and sugar contents. The pH value (5.28) was approximately that of the original roots.

The results of experiments conducted with 0.005 M KBr + 0.0005 M CaSO₄ (table 1) were practically identical with those of the 0.0005 M CaSO₄ solution. The respiratory quotients at the low oxygen tensions, 3.8 to 4.7 per cent, were again greater than one, the final sugar contents higher, and the ammonia + amide content depressed. The organic acid contents as before were not influenced by oxygen tensions as low as 3.8 per cent or as high as 85 per cent. At the lower oxygen tensions the respiratory activity along with absorption of KBr was greatly reduced. The general relationship between excess ca-

tion over anion absorption and organic acid formation held true even at the 3.8 per cent oxygen tension. At the 85 per cent oxygen tension there was no influence either on absorption of salt or on respiration over that of 20 per cent oxygen, but there was, as in the 0.0005 M CaSO_4 series, a lowering of the pH to 5.33.

In the oxygen tension study the possibility was considered that the absorption of nitrates might contribute to the oxygen supply necessary for respiration. The results in table 1 indicate that at 3.5 per

sulted in the formation of 8.6 milliequivalents per liter of organic acid.

In the 0.005 M KBr treated roots the milliequivalents of organic acids formed (7.2) approximated those of the excess cations absorbed (7.0), while, for roots placed in 0.025 M KBr solution, the cation excess was reduced to 5.1 milliequivalents per liter and 9.6 milliequivalents per liter of organic acids were formed. The ionic intake from the latter concentration as compared with 0.005 M concentration was almost doubled for the cations while the intake of anions was more than doubled. This increased absorption was not accompanied by a corresponding change in respiration. The actual increase in respiration was so slight that it is probably not significant. From this it may be inferred that the energy required for salt absorption was very small as compared to the total liberated in metabolism. The initial increase in respiration for the 0.001 M KBr solution was perhaps caused by a specific stimulation of metabolism by potassium bromide, but as more potassium bromide was absorbed, the initial influence on respiration diminished. The final concentrations of total sugar in the roots from the different KBr solutions were below those in the roots from the 0.0005 M CaSO_4 solution, although the differences may not be significant. The ammonia + amide content of the sap was not influenced significantly.

In the calcium bromide concentration study (fig. 7) the respiration of roots in culture solutions containing initially 0.0005 M CaBr_2 and 0.001 M CaBr_2 increased in comparison to those in 0.0005 M CaSO_4 solution. Decreases occurred in the 0.0025 M and the 0.0125 M solutions until in the latter salt concentration the oxygen absorbed by the roots was about equal to that taken up by those in the 0.0005 M CaSO_4 solution, while the carbon dioxide evolved was still significantly greater than in the absence of calcium bromide. The final sugar content in all the calcium bromide treated roots was significantly higher than in the roots treated with potassium bromide (compare fig. 7 with fig. 6) even though the CO_2 evolved in all cases except that of the 0.025 M solutions was approximately the same. Evidently, there is a fundamental difference in the metabolic products formed during the two salt treatments. Such a possibility was disclosed by Steward and Preston (1940) with potato discs in which potassium salts promoted protein formation in contrast with calcium salts. Another point of interest concerning calcium bromide absorption by barley roots is the reduction of the organic acids to minimum values (fig. 7). As is shown by the respiratory quotients, which are in these instances greater than one, this tendency is to release a considerable quantity of carbon as carbon dioxide which remained stored in the form of organic acids in the potassium bromide treated roots.

The relationship between cation and anion absorption from calcium bromide solutions merits particular attention. As far as could be ascertained from analyses of the external culture solution, there were no significant changes in the calcium content. How-

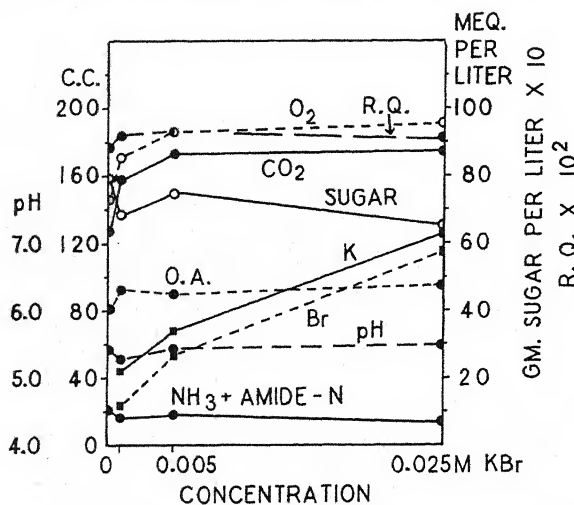


Fig. 6. Effects of potassium bromide concentration on the metabolic activity of excised barley roots maintained at 25.0°C. for eight hours. (K and Br values given as milliequivalents per liter of expressed sap. Other values as in figure 1.)

cent oxygen tension, there was an appreciable absorption of nitrate without great enhancement of respiration (compare with the similar KBr treatment given in table 1). The formation of organic acids was inhibited slightly at the lower oxygen tension, although the decrease may not be significant. In contrast with these results, the KNO_3 treatment with air increased respiration, the formation of organic acids and the use of sugar. Part of the oxygen for metabolism evidently was derived from the nitrates, since an increase in organic acids occurred without appreciably changing the respiratory quotient.

INFLUENCE OF SALT CONCENTRATION ON RESPIRATION AND FORMATION OF ORGANIC ACIDS.—Two salts, potassium bromide and calcium bromide, were used to study the influence of salt concentrations on respiration and the formation of organic acids. The results given in figures 6 and 7 indicate that initial concentrations of potassium bromide as low as 0.001 M have an effect on respiration almost as large as that of a 0.005 M solution (fig. 6). The quantity of cations absorbed from the 0.001 M KBr solution was approximately two-thirds, and the anion absorption was less than one-half of that in the 0.005 M KBr treated roots. The excess cation over anion absorbed equaled 10.5 milliequivalents per liter, and this re-

ever, the anion absorption was significant as shown by analyses of both the culture solution and the expressed sap. If the quantity of anions absorbed from the calcium bromide solution is contrasted with that absorbed from the potassium bromide solution, it will be found that the amounts in the respective saps are quite different. The quantity of anion absorbed from the potassium bromide solution increased with its external concentration, while the amount absorbed from calcium bromide solution remained nearly constant. This suggests some factor in the absorption mechanism which limits the intake of anions unaccompanied by cations. Perhaps this limitation is related to the extent of the organic acid anion decomposition, since the data in figure 7 indicate that the value for organic acids cannot be reduced under the present conditions below a certain minimum (33.2 to 40.2 meq.) in the presence of an ample supply of sugar. If the organic acid content of the original root material had been higher, it is possible that the anion absorption would have been greater. However, in the roots grown in the greenhouse by Hoagland and Broyer, in which the organic acid content is usually greater than in the roots grown in the dark by the present technique, the same relations exist between absorption of Br from KBr and CaBr₂. Possibly the rapid inward movement of K ions, as contrasted with Ca ions, accelerates the absorption of Br. This is a point requiring further investigation.

The data on absorption from calcium bromide solutions which show the effect on the pH of the expressed sap are of interest. The pH reaches a maximum value of 5.53 to 5.54 in all cases except in that of the 0.0005 M CaBr₂ solution for which it is 5.44. On the assumption that organic acids disappear when the plant cell tends to become more acid, the excess anion absorption should cause an increased acidity of the expressed sap. However, when it is considered that the dissociation constants of organic acids vary, it is possible that the acids that disappear or are reformed are those with high dissociation constants and consequently the acids that remain may actually lower the hydrogen ion concentration.

DISCUSSION.—The failure to influence the organic acid content of the excised barley roots by changes in temperature or oxygen tension is readily understandable from the viewpoint of the function of the acids in maintaining the acid-base balance of the cell sap. Neither changes in temperature from 5° to 35°C. or oxygen tensions of 3.2 to 88 per cent under the conditions of the experiments were influential in affecting the hydrogen ion concentration of the excised roots, and, consequently, there was no change in their organic acid content. The loss of organic acids occurring in an atmosphere of pure nitrogen may have been caused either by diffusion from injured cells or the counteraction of an increasing hydrogen ion activity.

The significant though relatively small increase in pH of expressed sap resulting from the calcium bromide treatment requires further consideration. According to the hypothesis here proposed, the ex-

cess anion absorption should effect a decrease in pH, which is in part counteracted by the decomposition of the organic acid anions, thus leaving the base in equilibrium with the absorbed bromide ions. With calcium bromide another factor may be involved in stabilizing the pH of the cell sap, namely, the decomposition of specific organic acid anions. If the organic acids having the highest dissociation constants were decomposed first, the remaining acids with lower degrees of dissociation would then tend to increase the pH. Analyses of the individual organic acids would be interesting from this standpoint.

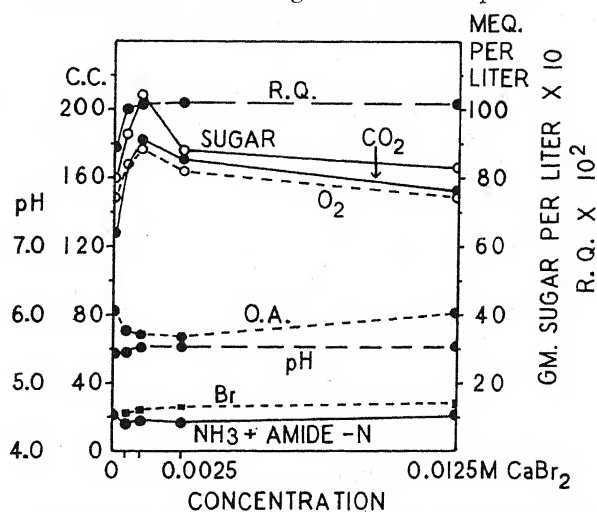


Fig. 7. Effects of calcium bromide concentration on the metabolic activity of excised barley roots maintained at 25.0°C. for eight hours. (For explanation of values see figures 1 and 6.)

It would be of interest to reinvestigate the influence of temperature, oxygen tension and light on the organic acids of plants from the viewpoint of the acid-base hypothesis presented in this paper. In such an investigation the influence of secondary factors should not be overlooked. A case in point is Wolf's (1931) observation that during the day, either in the light or dark, the decrease in organic acid content of leaves of *Sempervivum glaucum* and *Bryophyllum calycinum* is greatly inhibited by a gaseous environment high in carbon dioxide. At first glance this seemingly would cause a decrease in pH and therefore a decrease in organic acids, but Fife and Framp-ton (1935) have shown with sugar beet leaves, that carbon dioxide increases their ammonia content, and according to the acid-base hypothesis here proposed, organic acids would be formed when carbohydrates are available. Another field which might be investigated profitably from the present point of view is suggested by the conflict in the ideas of the Leipzig school and those of Bennet-Clark and of Vickery, *et al.*, regarding the origin of the organic acids. The views of the latter, as well as the present findings with excised barley roots, suggest that the organic acids arise from carbohydrates and not from amino acid decomposition as proposed by Ruhland and

Wetzel (1926). Perhaps the ammonia determined by the Leipzig workers was derived from labile amides, thus giving a false correlation of organic acid formation with ammonia. However, if there were an actual increase of ammonia under their conditions, then an increase in organic acids may have occurred to maintain the acid-base balance of their plants, assuming, of course, an adequate supply of carbohydrates.

The evidence presented here considers one function of organic acids to be the maintenance of the acid-base balance in the buffer system of the plant cells. This may not be their sole function as is amply indicated in the literature (Bennet-Clark, 1933a, b, c; Chibnall, 1939; Ruhland and Wolf, 1936; Vickery and Pucher, 1940). The organic acids may be involved in the respiratory cycle or even in photosynthesis. They may serve as precursors in the formation of amino acids or amides. In the absence of an adequate supply of carbohydrates they may act as a temporary source of energy. The present data indicate that when the organic acids serve to maintain the acid-base balance in the cells, the respiratory cycle is affected, judging by the accompanying changes in the respiratory quotient. When the organic acids disappear in the excised barley roots during excess anion absorption, the respiratory quotient increases, and, conversely, when cations are absorbed in excess of anions, the respiratory quotient decreases. The source of these acids must be dependent upon sugars either directly or indirectly, since the acids fail to form in the absence of avail-

able sugar, even though there are pronounced changes in the pH of the expressed sap.

SUMMARY

The total non-volatile organic acid content of excised barley roots containing an ample supply of available sugar remained constant unless a change in the ionic balance took place within the roots. When roots absorbed an excess of anions over cations, organic acids disappeared. Conversely, when cations were absorbed in excess of anions, organic acids were formed. The respiratory quotients reflected these organic acid changes by increasing as the acids disappeared and decreasing when they were formed.

Although the formation of organic acids in barley roots was dependent upon the presence of sugars, the acids were not in a dynamic equilibrium with them, since large decreases in total sugars failed to decrease the acid content of the roots except when the sugars had attained very low concentrations.

The failure to affect the total organic acid content of the excised roots by temperature or oxygen tension changes was attributed to the lack of influence of these factors on the ionic balance of the root cells.

There was no evidence to support the contention that the organic acids arose primarily from the oxidative deamination of amino acids. The indirect evidence indicates that the acids were derived from carbohydrates. ✓

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ADDITIONAL STUDIES REGARDING THE CATION ABSORPTION MECHANISM OF PLANTS IN SOIL¹

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NUMEROUS INVESTIGATIONS bear witness to the fact that the absorption of inorganic ions normally present in the soil is essentially concerned with the physiology of the living root system. (For reviews of this subject see Hoagland, 1936; *Annual Reviews of Biochemistry*, 1932-; Hoagland and Broyer, 1936.) Such information, gained from studies of nutrient solutions, is of great interest to practical agriculture; however, a major problem in the growing of crops in the field is presented by the fact that the mere presence in the soil of an ion does not necessarily insure its availability to the plant. The nutrient cations in the soil are for the most part adsorbed on the surfaces of colloidal particles. Their release from the particles must occur in accordance with the laws of colloidal chemistry. For this reason a careful study of the mechanism by which the plant root effects this release would seem to be of great practical as well as theoretical importance. This paper purports to deal with certain aspects of this problem.

STUDIES WITH SOLUTION CULTURES.—Valuable information relative to the mineral requirements of plants has been obtained with the use of specially prepared nutrient solutions. Moreover, with this technique, the rates of absorption of ions from solutions by roots have been determined and numerous factors affecting the rates have been investigated (Hoagland and Broyer, 1936; Broyer and Overstreet, 1940).

Owing to the fact that with all suspensions we have to deal with an intermicellar as well as a micellar region, the solution culture studies constitute a reasonable starting point for the study of ion absorption from colloidal clay suspensions.

The present experiments are largely restricted to the investigation of the absorption of potassium by "low-salt"² barley roots. This ion, when present in solution cultures in association with monovalent

anions such as chloride, bromide and bicarbonate, is perhaps the one most rapidly absorbed by the experimental material. The rate of absorption is dependent within somewhat narrow limits on the concentration of potassium at the root surface. This fact will be evident from the following experiment.

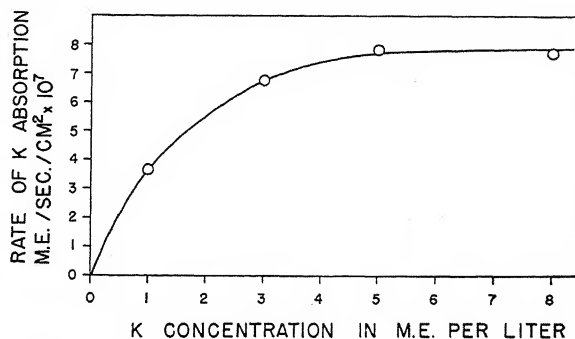


Fig. 1. Rate of potassium absorption by excised barley roots as influenced by the concentration of potassium in the culture medium. Segments from the actively growing regions of barley roots were exposed for twelve minutes to culture media of concentrations from 1 to 8 milliequivalents per liter of total potassium as KCl. Radioactive K was employed as an indicator of the potassium absorbed.

From three-week-old barley roots, grown according to the method of Hoagland and Broyer (1936), unbranched segments 1 cm. in length were selected from regions within 4 cm. of the root tip. The mean diameter of the segments as measured with a micrometer was found to be 0.059 ± 0.003 cm. Samples comprising 120 segments each were exposed to flowing culture solutions containing radioactive potassium as KCl. At the conclusion of the absorption period (twelve minutes), the samples were ashed and the K absorbed determined by counting the ash with the Geiger-Müller counter. The mean rate of potassium absorption per unit root surface was calculated for the twelve-minute period, the approximate surface having been calculated from the measured diameter and length of the segments. The variation of absorption rate with concentration is given in figure 1. As may be seen from the graph, the absorption rate above a normality of 0.005 is evidently independent of concentration. Between the normalities of 0.005 and zero the rate diminishes rapidly. The maximum rate of potassium absorption was approxi-

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The authors desire to express their appreciation to Professors D. R. Hoagland and H. Jenny for their kind suggestions in connection with this problem. We are indebted to the Radiation Laboratory of the University of California for the radioactive potassium.

² The term "low-salt" implies that the plants during their growth period were limited with respect to their supply of essential nutrient salts (see Hoagland and Broyer, 1936). Roots of this type are also high in carbohydrate.

mately 7.8×10^{-7} milliequivalents per second per cm^2 . Since the fresh weight of 120 segments was found to be 0.365 gm., this rate is equivalent to 1.71 M.E. potassium per hour per 100 gm. fresh roots, which is a much higher rate than that usually observed for the whole root system (about 0.5 M.E. potassium per hour per 100 gm. fresh roots). Similar results have been reported by Prevot and Steward (1936).

It is of interest to compare the rate of potassium absorption per unit surface with the rate at which

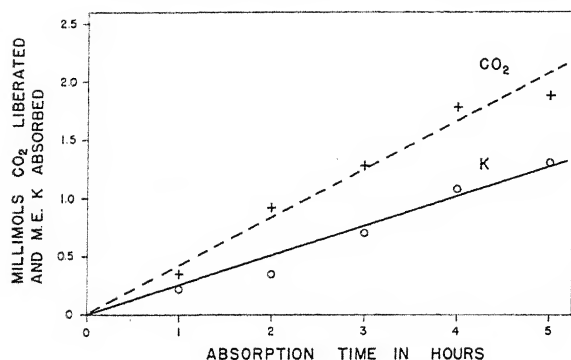


Fig. 2. Comparison of the amounts of carbon dioxide liberated from and potassium absorbed by excised barley roots with time. Composite samples of barley roots were exposed for time periods of from one to five hours to colloidal K-bentonite suspensions, continuously aerated with a stream of CO_2 -free air.

the potassium ions collide with the surface. An approximate value for the number of collisions per unit time per unit surface may be calculated from the perfect gas laws. On the basis of this calculation, the value for a normality of 0.005 is approximately 10^{21} hits per second per cm^2 . When this value is compared with the corresponding absorption rate of 4.7×10^{14} ions per second per cm^2 , it becomes apparent that only about 10^{-5} per cent of the potassium ions striking the root surface are absorbed at this concentration.

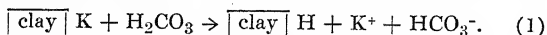
STUDIES WITH CLAY SUSPENSIONS.—As has been discussed elsewhere (Overstreet and Jenny, 1939) all experimental evidence points to the fact that the composition of the intermicellar liquid in a clay suspension is not measurably different from that of the filtrate or centrifugate. When a suspension of a freshly electrolyzed hydrogen bentonite is titrated with KOH or NaOH to pH 7, potassium or sodium clay suspensions are obtained that yield filtrates very nearly devoid of electrolytes. For example, the filtrate from a 1.5 per cent potassium bentonite suspension (10 M.E. K per liter) prepared in this way was found to have a pH value of 7.3 (0.5×10^{-7} molal $[\text{H}^+]$). Thus the potassium concentration in the intermicellar liquid must have been of the order of 10^{-7} molal. On the basis of the data of figure 1, one would expect that accumulation of K by roots from such a solution would be extremely slow, even though the concentration of the solution

were constantly maintained. However, in apparent contradiction to this reasoning, barley roots readily absorb potassium or sodium from the homionic clay suspensions. With suspensions containing 1–5 M.E. per liter of adsorbed K the rate of K absorption by the plant has been found to be 25 to 50 per cent of that from comparable salt solutions. In the case of sodium clay suspensions in this concentration range, the absorption may even be greater than from the corresponding salt solutions (compare Overstreet and Jenny, 1939).

A number of explanations of this perplexing phenomenon have been proposed (Comber, 1922; Breazeale, 1923; Truog, 1927; Jenny and Overstreet, 1938). In the present instance we shall concern ourselves with the two most plausible explanations. The first of these involves the carbonic acid which is excreted by the roots during respiration. The second is based on the contact mechanism proposed by Jenny and Overstreet (1938).

The CO_2 hypothesis.—Barley roots continuously evolve CO_2 during ion absorption. The processes occurring within the plant requisite for active electrolyte accumulation (for example, the oxidation of sugars to organic acids and CO_2) invariably result in the excretion of carbonic acid. This fact is made clear from the following absorption-time curve for barley roots in K-bentonite suspensions (fig. 2). In obtaining each point on the curve, 5 gm. of excised barley roots were immersed in 300 ml. of a 1.45 per cent K-bentonite suspension which contained 10 M.E. K per liter. During the absorption period the suspension was aerated with CO_2 -free air. The outgoing air was led through a NaOH tower for the purpose of absorbing the evolved CO_2 . At the conclusion of each absorption period both the NaOH from the tower and the suspension were analyzed for CO_2 by means of the Warburg manometric method. The roots were washed, ashed and analyzed for K by means of the cobalti-nitrite method. The results were compared with K analyses on the original root material. The net potassium absorption and the total carbonic acid liberation were expressed as millimols per 100 gm. fresh roots. Inspection of the figure reveals that the K absorption and CO_2 liberation are nearly straight line functions of the time. As may be calculated from the graph, 1.8 millimols of CO_2 were given off by the roots for each millimol of potassium absorbed. This result is in harmony with previous experiments with clay suspensions wherein it has been observed that 1–2 millimols CO_2 were respired per millimol of K absorbed.

Inasmuch as the excretion of H_2CO_3 invariably accompanies the accumulation of ions by roots, it has seemed reasonable to many investigators to assume that the initial step in ion absorption by roots from clay suspensions is the release of ions adsorbed on the clay particles to the intermicellar liquid by an exchange for the hydrogen of carbonic acid. For the case of a potassium clay this process may be indicated as follows:



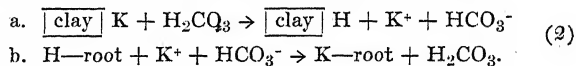
The second step in the accumulation process is, according to one view, the absorption of K^+ and HCO_3^- as ion pairs from the intermicellar liquid. However, appreciable accumulation by roots of HCO_3^- , as such, has never been demonstrated. In a recent study using radioactive carbon, Overstreet, Ruben and Broyer (1940) found that in the case of a culture solution of 0.001N $KHCO_3$, a small fraction of the HCO_3^- was apparently reduced by the roots with the formation in the tissue of compounds of high molecular weight. An extremely small fraction of the HCO_3^- was found unchanged within the root system. Moreover, it was found that the maximum number of equivalents of HCO_3^- utilized and retained in various carbon forms by the root system was far less than the number of equivalents of K^+ absorbed. This result is supplemented by absorption studies carried out in this laboratory (compare Ulrich, 1941) wherein a complete ionic balance is obtained on the culture solution and on the root sap following a period of active electrolyte absorption by the roots. The results of these studies indicate that an excess accumulation of cations over anions is compensated in the culture solution by hydrogen ions or other ions derived from roots (*e.g.* Ca) and in the root system by organic acid anions. The extension of this type of study to colloidal clay suspensions is embodied in the following experiment.

Five samples of "low-salt" excised barley roots (approximately 100 gm. fresh weight) were each immersed in 3,000 ml. of culture medium. The media comprised three salt solutions and two K-bentonite suspensions. The culture media were continuously aerated with a stream of CO_2 -free air for a period of five hours. The respired CO_2 was collected as before in NaOH towers. At the conclusion of the absorption period, the roots were thoroughly washed, weighed and frozen. The sap was then expressed from the roots by means of an hydraulic press. The press-cakes were dried at $80^\circ C.$ and weighed. The expressed saps were analyzed for Ca, Mg, K, Na, Br, SO_4 , PO_4 , Cl and organic acids. The results of the chemical analyses were expressed in terms of milliequivalents in the total sap from 100 gm. of fresh roots. On this basis it was found that the roots from the distilled water control contained 0.42 M.E. Ca^{++} , 0.19 M.E. Mg^{++} , 1.915 M.E. K^+ , 0.45 M.E. Na^+ , 0.00 M.E. Br, 0.45 M.E. SO_4^{--} , 0.43 M.E. PO_4^{--} , 0.06

M.E. Cl^- , and 3.26 M.E. organic acids.³ The respiration of 100 gm. of the control roots was 3.08 millimols CO_2 . The respiration and net gains or losses of the various ions as compared with the composition of the control roots are given for each sample in table 1.

The column denoted " Δ cations" represents the net excess of cations over anions which was accumulated in the sap in the five-hour period. The column " Δ organic acids" represents the increase in organic acids as determined by the method of Isaacs and Broyer (1942). Under the conditions of the experiment, " Δ organic acids" corresponds approximately to the increase in organic anions other than HCO_3^- . As may be seen from the table, the excess accumulation of cations over anions is roughly balanced by organic anions (other than HCO_3^-) which are synthesized within the plant. Moreover, it is apparent that these synthesized organic acids are the ultimate source of the hydrogen which replaces the adsorbed potassium on the clay and not carbonic acid. We are not now attempting consideration of the case of equivalent absorption of cations and anions (*e.g.* K and Br ions) by the plant.

The above findings would seem to indicate that carbonic acid is not involved in the end results of the accumulation reaction. The experiments, however, do not rule out the possibility that carbonic acid may be involved in an intermediate step in the process. For example, one might postulate that the absorption of potassium from clay suspensions takes place by means of the following two steps:



On the assumption of this mechanism the H_2CO_3 can be regarded as a carrier of K^+ between the clay and root surfaces.

The available experimental evidence necessitates ascribing to carbonic acid an intermediate rôle in the cation absorption process in clay systems. Whether or not its direct function as a carrier is important is the subject of current researches in this laboratory. However, it should be pointed out that such a mechanism as outlined in (2) will by no means account for

³ This value includes organic acid anions and undissociated organic acids. For this reason, a balance between total cation and anion is not readily made. Further, the cation total should include H^+ and organic bases.

TABLE 1. Comparison of the amount of carbon dioxide required by and the net change in electrolyte content of composite samples of barley roots, when exposed to various media.

Culture medium	K conc. M.E./liter	Respiration	δ^a Ca	δ Mg	δ K	δ Na	δ Br	δ SO_4	δ PO_4	δ Cl	Δ Cations	Δ Organic acids
		m. mols CO_2										
K Br	5	3.69	0.0	0.0	2.3	0.0	1.3	0.1	0.0	0.0	0.9	0.9
$KHCO_3$	5	1.34	0.0	0.0	2.7	0.0	..	0.1	0.0	0.0	2.6	2.1
K_2SO_4	5	2.61	0.0	0.2	1.7	0.0	..	0.3	0.0	0.0	1.6	1.6
K-clay	5	2.55	0.0	0.1	1.5	0.1	..	0.1	0.0	0.0	1.6	1.5
K-clay	5	2.51	0.0	0.2	1.1	0.0 ^b	0.0	0.0	1.3	0.9

^a The individual and summed differences (δ and Δ) are expressed in milliequivalents.

^b Not determined.

certain experimental observations; for it is inconceivable that the concentration of bicarbonates in the intermicellar liquid will at any time be higher than in the suspension when saturated with CO_2 at one atmosphere pressure. As already noted, recent investigations by Overstreet and Jenny (1939) concerning the absorption of sodium from sodium bentonites revealed that the rate of Na^+ absorption from the intermicellar liquid of the CO_2 -saturated suspension was at all concentrations much less than the rate of absorption from the original suspension.

The contact hypothesis.—The inorganic cations in soil and prepared clay suspensions are for the most part adsorbed on the surfaces of the colloidal particles. The contact hypothesis is based on the fact that these ions, although held in the adsorbed state, are capable of entering into certain chemical processes. While abundant experimental support for this fact is at hand, the theory of the processes cannot be treated rigidly by means of thermodynamics, since the quantities involved (for example, the electric potential in the neighborhood of a charged colloidal surface) are incapable of thermodynamic definition. An extra-thermodynamic interpretation of the problem, along the lines of that used by Debye and Hückel for the case of strong electrolytes, will be published elsewhere. The qualitative aspects of the contact theory may be presented with the adoption of the idea of a diffuse ion swarm about each particle. On this basis the adsorbed ions move rapidly about in a region near the external surface of the particle, much as O_2 molecules move about in the earth's atmosphere. The mean concentration of the ions as a function of the distance from the particle wall depends, among other things, on the particle charge and on the mean concentrations of anions and cations in the system. In the K-clay suspensions discussed above, the concentration of K ions presumably reaches a constant low value at a distance of a few hundred Ångströms from the particles.

With the aforementioned picture of the adsorbed ions, Jenny and Overstreet (1938) have envisaged two distinct types of phenomena occurring in colloidal systems.

The first phenomenon has to do with the ion swarm about a single surface. If by some process a particular ion species is introduced at a given point in the ion swarm, it would be expected to distribute itself in a definite manner throughout the entire ion swarm. This reaction has been termed "the surface migration of adsorbed ions."

The second phenomenon concerns two distinct surfaces having ion swarms which approach very close to one another. In this case the ion swarms would be expected to intermingle. On separation of the surfaces, it would be found that ions from the swarm of the one surface had been transferred to that of the other surface and *vice versa*. This process has been designated as "contact exchange of ions." In this process a transfer of ions from one surface to another takes place which is essentially independent of the intermicellar liquid. On the basis of this mech-

anism Jenny and Overstreet have sought to interpret the absorption of cations by plant roots from soil colloids. By postulating that the root itself possesses an ion swarm about its surface (which cannot now be defined with precision) they were thus able to explain the initial step in cation absorption from soils without resorting to assumed intermediate reactions involving carbonic acid.

In conclusion, it should be pointed out that the two hypotheses outlined in this paper are not necessarily in opposition. The mechanisms are not mutually exclusive. The carbonic acid hypothesis represents an approach to the problem of availability in soils by way of studies with artificially-prepared nutrient solutions. In several respects this approach is justified; for example, certain nutrient anions such as NO_3^- and SO_4^{2-} are contained exclusively in the intermicellar liquid or soil solution. The contact mechanism, on the other hand, is an interpretation of the problem from the viewpoint of surface chemistry. This approach was prompted by a realization of the general inadequacy of solution culture and soil solution studies for the estimation of nutrient availability in the soil system as a whole. While it seems established that the carbonic acid theory alone will not explain certain observable phenomena, the question whether or not the contact theory can withstand the same test must await further experimentation.

SUMMARY

With the use of flowing K Cl culture solutions containing radioactive K, a determination of the influence of concentration on the rate of potassium absorption by barley roots was made for very short time intervals. An approximate value for the maximum absorption rate per unit area of root surface is calculated for the more active regions of the tissue.

A determination was made of the magnitude of the carbonic acid liberation by absorbing roots in K-bentonite suspensions as a function of time.

The relationship between organic acid synthesis by roots and ion absorption from K-bentonite suspensions and potassium salt solutions was investigated. It is concluded that carbonic acid, if involved at all, must play an intermediate rôle in cation absorption from clay suspensions.

Proposed mechanisms for cation absorption by roots in soil and clay suspensions are discussed.

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THE SIGNIFICANCE OF X-RAYS IN STUDYING THE ORIENTATION OF CELLULOSE IN THE SECONDARY WALL OF TRACHEIDS¹

I. W. Bailey and Earl E. Berkley

MUCH EVIDENCE has accumulated from varied lines of research which indicates that the orientation of cellulose fluctuates more or less conspicuously in passing from the first-formed to the last-formed part of the walls of plant cells. In the case of normal tracheids of gymnosperms and dicotyledons, the secondary wall is commonly a three-layered structure; the first-formed and the last-formed lamellae of this wall have cellulose that is transversely oriented or arranged in helices of relatively low pitch, whereas the intervening lamellae have cellulose that is oriented longitudinally or in helices of comparatively steep pitch.

Although admitting changes of orientation in the cell wall of *Valonia* and of various tissue cells, Preston (1934, 1939) maintains that there is but a single spiral arrangement of cellulose throughout the secondary wall of coniferous tracheids. The chief evidence presented in support of such a divergent view consists of a small photograph obtained by X-raying a tracheid isolated from wood by maceration. Neither this photograph nor its enlargement exhibit a clearly defined diffraction pattern.

In the case of cotton, ramie and other plant fibers, significant data are obtained by X-ray analyses of aggregates or bundles of cells. Thus, in view of the fact that the structurally simpler types of wood are composed largely of longitudinally oriented tracheids, it is reasonable to anticipate that pertinent data may be obtained by X-raying small pieces of secondary xylem. That such is indeed the case has been demonstrated by Stillwell (1933), Sisson (1935, 1938) and others. It should be emphasized in this connection, however, that in selecting and preparing material for X-ray analysis and in interpreting diffraction patterns, it is essential to be fully cognizant of the numerous morphological, histological, chemical and other variables in the material that is

being studied. For example, certain serious misconceptions concerning the composition of the primary wall of the cotton hair might have been avoided by an accurate visualization of the significance of such variables (Berkley, 1939).

It is advisable, therefore, before discussing X-ray analyses of wood to evaluate the evidence that has accumulated from varied lines of research regarding the visible structure of the secondary wall of tracheary cells.

VISIBLE STRIATIONS AND FIBRILLAR ORIENTATIONS IN THE UNSWOLLEN WALLS OF TRACHEIDS.—Preston (1934) states that "in general no indication is given, visible under an ordinary microscope, of the direction of the cellulose fibrils in the walls" of tracheids. This is an untenable premise. Striations, such as are characteristic of *Valonia* and of various tissue cells, are visible in the secondary walls of many tracheids, provided that the cells are not mounted in media of unfavorable indices of refraction. Freshly cut, moist, longitudinal sections of the wood of living trees fortunately are suitable for microscopic examination and may be studied without staining, chemical pretreatments, or the use of mounting media. Detailed investigations of such sections demonstrate that the first-formed and the last-formed parts of the secondary wall have striations that are transversely oriented to the long axis of the cells or are arranged in helices² of relatively low pitch (45° – 90°), whereas the intervening part of the wall exhibits striae that are longitudinally oriented or arranged in helices of comparatively steep pitch (0° – 45°). As in *Valonia* and other plant cells, these striations are due fundamentally to the fibrillar structure of the cellulosic

² The term, helical, is used in a descriptive sense without rigid geometrical implications. The tracheids of conifers rarely are perfectly cylindrical, but tend to have more or less conspicuously flattened sides. The pitch of the helix changes not infrequently in passing from one surface of the cell to another.

¹ Received for publication November 5, 1941.

matrix and may be differentiated without difficulty from slip planes, cracks, pressure ridges, parallel scratches and other abnormalities mechanically induced by the edge of a knife in sectioning.

It is evident, accordingly, that sections from freshly cut wood of living trees may be utilized as controls or bases of comparison in studying the effects of various chemical and other treatments upon the orientation of the cellulosic fibrils in tracheary cells. When such sections are immersed for a few minutes in chlorine water, stained in iodine-potassium iodide and mounted under a cover glass in a

crystals are equally conspicuous in transverse, longitudinal and diagonal sections of wood, they provide a useful and reliable means of studying fluctuations in the orientation of cellulose in the successively formed parts of the secondary wall. Evidence that may be obtained by this technique cannot be brushed aside upon the mere assumption that the deviations in orientation are induced by swelling. In the first place, the crystals form in dilutions of sulphuric acid—50 per cent or less—which produce no detectable swelling in heavily lignified cell walls. In the second place, the visible striae in untreated sections have

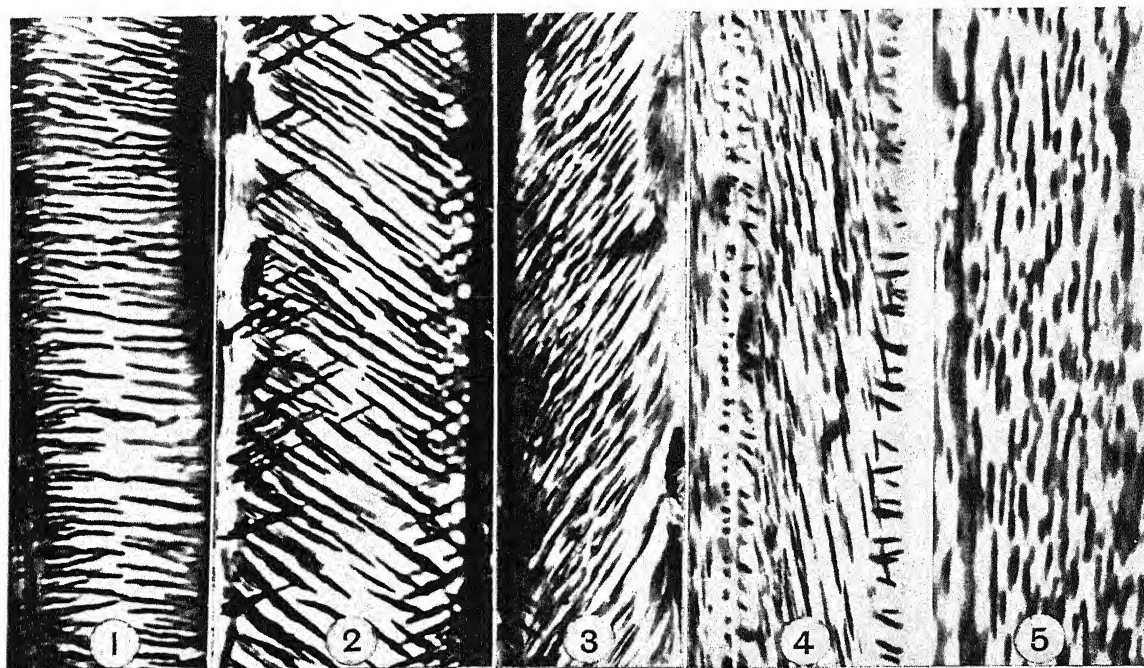


Fig. 1-5. Orientation of crystals in the outer and central layers of the secondary walls of tracheids.—Fig. 1. Transverse orientation in outer layer. $\times 840$.—Fig. 2. Pitch of 60° in outer layer. $\times 840$.—Fig. 3. Pitch of 40° in central layer. $\times 840$.—Fig. 4. Pitch of 10° in central layer. $\times 750$.—Fig. 5. Longitudinal orientation in central layer. $\times 1,560$.

small drop of diluted sulphuric acid, crystals of iodine may be induced to form in the elongated porosities of the cellulosic matrix (Bailey and Vestal, 1937a). The long axis of these slender crystals³ is oriented parallel to the long axis of the porosities and, therefore, *pari passu* to the long axis of the fibrils. In other words, where the visible striae or fibrils are longitudinally oriented, the crystals are arranged parallel to the long axis of the cell (fig. 5); where the striae are transversely oriented, the crystals are arranged at right angles to the long axis of the cell (fig. 1), and where the striations have a helical orientation, the crystals are arranged in a helix of identical pitch (fig. 2-4). Since the dark-colored

³ The size and the number of the crystals may be altered by varying the details of the technique. Numerous minute crystals are desirable for accurate visual study of the fibrillar orientations in adjacent lamellae, but larger crystal complexes are advantageous for photographic illustration (fig. 1-5).

similar orientations. In the third place, even the swelling produced by stronger acid—50 per cent to 60 per cent—does not induce changes of from 45° to 90° in the fibrillar orientations of the inner (last-formed) and outer (first-formed) layers of such heavily lignified secondary walls. Nor can the swelling produce abrupt transitions in adjacent lamellae from right-handed to left-handed helices or *vice versa*.

EFFECTS OF DRYING UPON FIBRILLAR ORIENTATIONS.—Many investigations in the past have been based upon the study of cells or tissues that at some stage have been dried below the fiber saturation point. For example, the customary procedure in preparing fibers for X-ray analyses involves a thorough drying. Both primary and secondary walls contract in drying along planes at right angles to the long axis of the fibrils. The contraction of the walls, in turn, produces more or less extensive changes in the

form and the dimensions of the cells; the type and the magnitude of which depend upon a wide variety of factors. Obviously the orientation of the fibrils may be modified during the process of drying. Therefore, microscopic observations and X-ray analyses of desiccated material provide reliable evidence regarding the orientation of fibrils, crystallites and chain molecules in *dry* specimens, but do not *necessarily* provide an accurate picture of the orientations in cells that have never been dried below the fiber saturation point. It is essential in each particular case to obtain some clue regarding the changes that may have occurred during drying.

Longitudinal sections of the freshly cut wood of living trees may be mounted under a cover glass and kept under continuous microscopic observation throughout the entire process of desiccation. As the sections dry, the visible striations of the secondary wall of the tracheids become increasingly conspicuous. In the case of the normal wood of coniferous species and of the vesselless dicotyledon, *Trochodendron*, there are minor readjustments during drying, but there are no extensive changes in the orientation of the fibrils except possibly in the corners of rectangular or hexagonal cells. Therefore, X-ray analyses of wood which has not become excessively warped or deformed during drying should yield fairly close approximations to the orientations of cellulose in unseasoned wood.

EFFECTS OF DELIGNIFICATION AND MACERATION UPON FIBRILLAR ORIENTATIONS.—Numerous investigations in the past have been based upon the microscopic study of pulp or of cells that have been isolated by maceration. Furthermore, in obtaining sharply defined X-ray diffraction patterns of the cellulose in wood, it is necessary to remove most of the non-cellulosic constituents, particularly the lignin. Therefore, it is essential to determine what effects delignifying and macerating reagents have upon cellulose. In the case of the woods that were selected for investigation, it is possible to delignify and macerate longitudinal sections and to study the microscopically visible effects of specific reagents upon the fibrillar orientations of the cellulose. As in drying woods, minor readjustments of form and structure occur during delignification⁴ and maceration, but there are no pronounced changes in the fibrillar orientations. Walls having layers with transverse, longitudinal, right-handed or left-handed helical orientations, retain these orientations throughout the processes of delignification and of maceration. This is of considerable significance since it justifies the study of fibrillar orientations in single cells or in pieces of cell wall. Thus, the complications of accurately focusing through adjacent cells or cell walls may be avoided. It indicates, in addition, that data obtained by the micro-dissection of isolated cells (Seifriz and Hock, 1936) cannot be ignored or rejected upon the

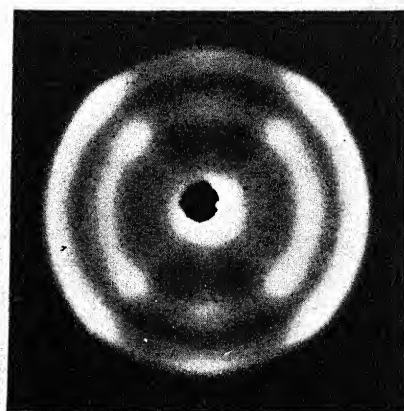
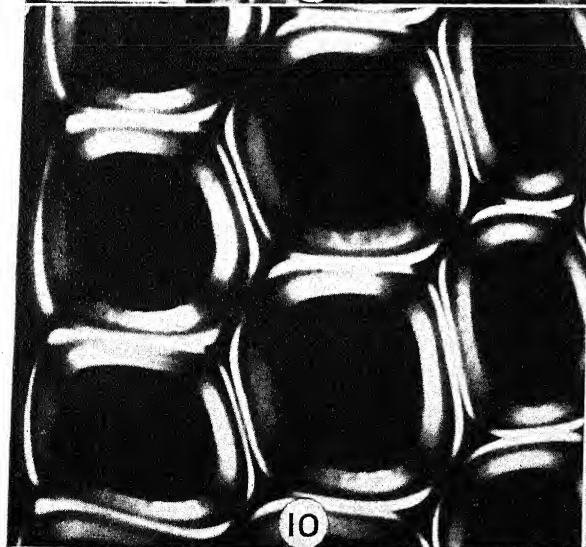
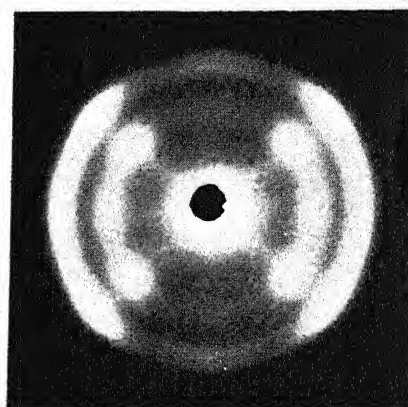
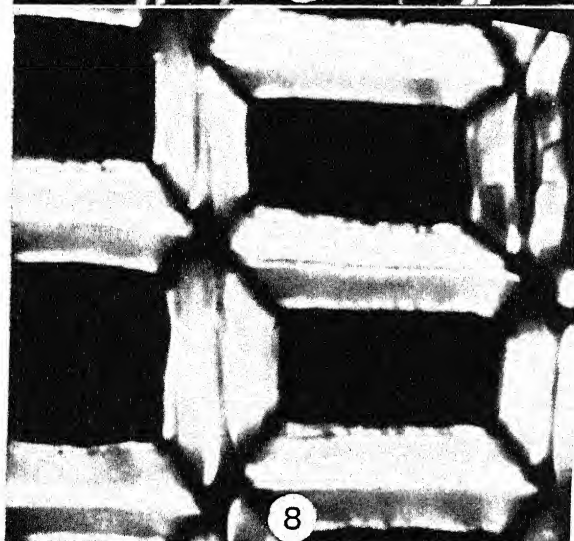
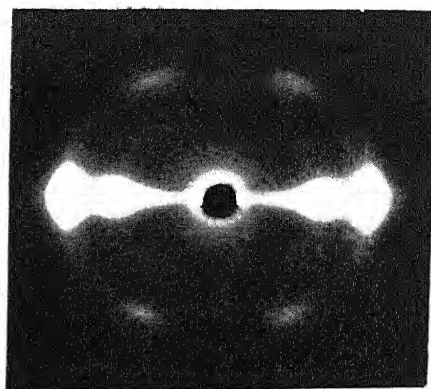
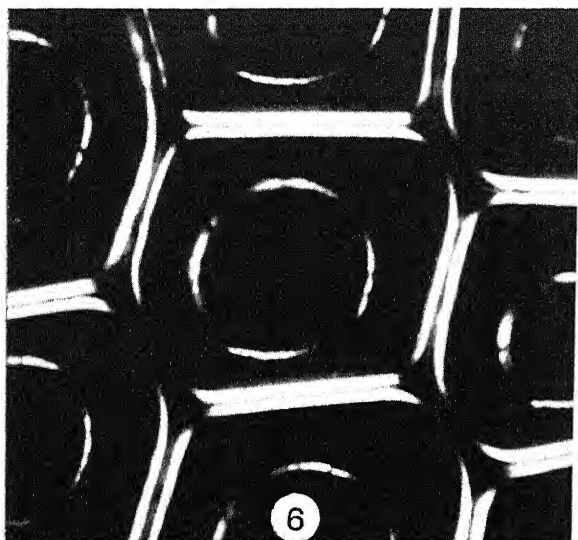
⁴ That the removal of lignin and cellulose from wood causes no fundamental change in the X-ray diffraction pattern of cellulose is indicated by the work of Preston and Allsopp (1939).

assumption that the fibrillar orientations were extensively modified during maceration.

CORRELATIONS BETWEEN FIBRILLAR ORIENTATIONS AND ANISOTROPY.—Physical evidence has accumulated in recent years which indicates that the chain molecules and crystallites of cellulose are oriented within fibrils with their long axes parallel to the long axis of the fibrils. Isolated macro-fibrils exhibit positive optical anisotropy and sharply defined extinction angles. Thus, the optical anisotropy of the various layers of the cell wall should correlate closely with the visible fibrillar orientations within them. Where the striae or fibrils of the central layer of the secondary wall of tracheids are longitudinally oriented, this layer should exhibit no birefringence under the polarizing microscope in transverse sections of the wood; it should show intense birefringence in longitudinal sections, and more or less reduced birefringence in sections cut at varying diagonals to the long axis of the cell. Conversely, where the fibrils of the inner and outer layers are oriented at right angles to the long axis of the cell, these layers should exhibit intense birefringence in transverse sections of wood, more or less reduced birefringence in diagonal sections, and no birefringence in those parts of longitudinal sections where plane surfaces of the walls can be viewed edge on. Where the layers have helical orientations, they should be more or less birefringent in both transverse and longitudinal sections, depending upon the pitch of the helix, and should exhibit no birefringence on one side of the cell in certain specific planes of diagonal section. Where a layer has a helical orientation of approximately 45°, this layer should be dark on one side of the cell and intensely birefringent upon the opposite side in sections cut at an angle of 45° to the long axis of the cell.

All of these theoretical predictions have been fully verified in woods of conifers and dicotyledons (Bailey, 1940) but there are certain of them—those involving absence of birefringence—which require unusually favorable material and great care in sectioning for convincing proof. To demonstrate the absence of birefringence from longitudinally striated layers⁵ in transverse sections of wood, it is essential in the first place to find specimens in which the tracheids are straight and are uniformly oriented with their long axis parallel to the vertical axis of the tree. Such specimens are by no means of common occurrence. In the second place, the plane of sectioning must be oriented with precision at right angles to the long axis of the tracheids and the sections must be cut with a very thin, perfectly smooth and properly oriented knife edge which does not produce serious distortions of the fibrils in sectioning. In the case of layers having fibrils arranged in planes at right angles⁵ to the long axis of the tracheids, it is essential to obtain accurately oriented longitudinal sections from wood composed of large tracheids with flattened parallel

⁵ Wall layers having a perfectly uniform orientation of fibrils throughout all of their constituent lamellae are of exceptional, rather than of typical, occurrence in tracheids.



sides. In such sections only is it possible to eliminate the complicating effects of wall curvatures. It should be noted in this connection that no critical evidence can be obtained under the polarizing microscope from sections of the quality illustrated by Preston (1939).

Cellulose swells considerably in planes at right angles to the long axis of the fibrils, but only slightly in a plane parallel to this axis. Therefore, if the inner and the outer layers of the secondary wall of the tracheids have fibrillar orientations which deviate markedly from that of the central layer, these layers should exhibit characteristic and predictable differences in swelling anisotropy. Where the fibrils of the outer layer are transversely oriented or are arranged in helices of low pitch—i.e., nearly at right angles to those of the central layer—the outer layer should not increase in circumference during swelling, but should act as a constricting cylinder about the laterally expanding central layer. Numerous evidences of this constricting behavior of the outer layer may be observed in the swelling of isolated tracheids and of transverse sections of wood (Bailey, 1940).

CORRELATIONS BETWEEN FIBRILLAR ORIENTATIONS AND PLANES OF ENZYMATIC HYDROLYSIS.—There are certain ubiquitous fungi whose hyphae penetrate and move forward within the secondary walls of tracheary cells. Enzymatic hydrolysis progresses along two sets of predetermined planes, one of which is oriented parallel to the long axis of the fibrils of cellulose (Bailey and Vestal, 1937b). The hyphae and the elongated cavities which they produce are oriented with their long axes parallel to the long axis of the fibrils. Where the hyphae penetrate the inner and the outer layers of the secondary walls of tracheids, they are oriented transversely or in helices of low pitch, whereas in the central layer of such walls, they are oriented longitudinally or in helices of comparatively steep pitch with respect to the long axis of the tracheids.

UNRELIABLE INDEXES OF FIBRILLAR ORIENTATIONS.—Various investigators have attempted to determine fibrillar or micellar orientations by measuring the extinction angles of cell walls in surface view. Such measurements lead to serious misconceptions, however, when derived from walls that are composed of two or more layers of fluctuating thicknesses and different fibrillar orientations. The problem is further complicated in the case of many tracheids where the orientation of the fibrils fluctuates considerably in the successively formed lamellae of the individual layers of the secondary wall or even within different parts of the same lamellae.

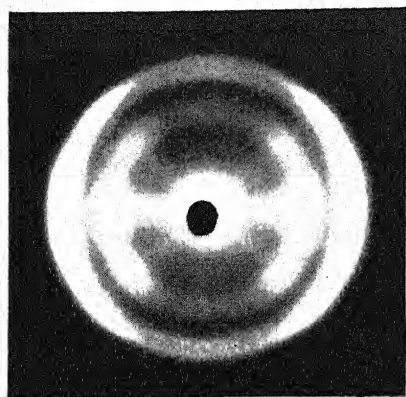
In the case of thick secondary walls of the three-layered type, the long axis of the slitlike pit orifices is frequently oriented approximately parallel to the fibrillar orientation of the central layer, but affords

no evidence regarding the fibrillar orientations of the inner and outer layers of the cell wall. In thin-walled tracheids, the pit orifices commonly afford no reliable clues concerning the fibrillar orientations of any of the wall layers. Similarly, planes of mechanical cleavage may afford useful evidence in the case of radio-longitudinally or radio-helically striated layers, but are difficult to interpret accurately in the case of other types of wall layers.

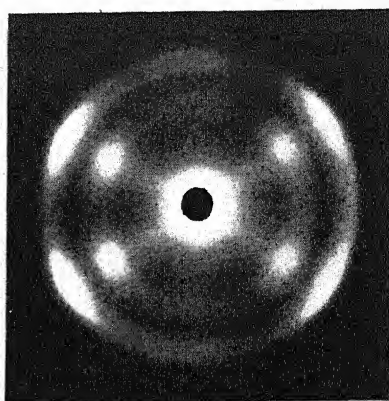
CONCLUSIONS REGARDING VISIBLE ORIENTATIONS.—It is evident from the preceding discussion that the fibrillar orientations of cellulose fluctuate more or less markedly in passing from the first-formed to the last-formed lamellae of the secondary walls of tracheids, and that the most reliable means at present of demonstrating such deviations in orientation are microscopically visible striations and crystals that may be induced to form in the elongated porosities of the cellulosic matrix. The close correlation between fibrillar orientation, optical anisotropy, swelling anisotropy, and predetermined planes of hydrolysis supports the view that the chain molecules and crystallites of cellulose are oriented within fibrils with their long axes parallel to the long axis of the fibrils. If the chain molecules are so oriented in the fibrils of tracheids, it should be possible under favorable circumstances to correlate salient features of X-ray diffraction patterns with visible fibrillar orientations.

SELECTION AND PREPARATION OF WOOD FOR X-RAYING.—In obtaining X-ray diffraction patterns from wood, it is essential to reduce the number of morphological, histological, chemical and other variables to a minimum, and in any case to obtain reliable evidence regarding the significance of such variables in the material that is being analyzed. In dealing with tracheids it is advisable to use wood which is devoid of vessels and contains a low percentage of parenchyma. Straight-grained secondary xylem from the outermost part of the stems of large conifers and of the vesselless dicotyledon, *Trochodendron*, provide favorable material for X-ray analyses. In such specimens, the tracheids are of large size, thus reducing the ratio of tapered ends within the limits of a unit area. Furthermore, the tracheids tend to be arranged in more uniform serialations, and the curvature of the growth rings is greatly reduced. The problems of X-ray analysis may be further simplified by separating the early wood from the late wood. To facilitate this procedure, it is desirable to select wood having very sharply and abruptly defined layers of dense late wood. However, before dissecting small cubes of wood into their constituent layers of early wood and late wood, it is essential to cut transverse, radial and tangential sections for microscopic study and as permanent records of the specific combinations of

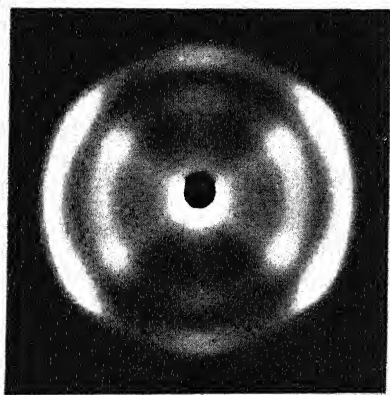
Fig. 6-11.—Fig. 6, 8, 10. Transverse sections of wood, photographed in polarized light between crossed Nicols.—Fig. 7, 9, 11. Corresponding X-ray diffraction patterns of delignified wood with beam oriented parallel to the long axis of the wood rays.—Fig. 6. *Pinus longifolia*, late wood. $\times 1,050$.—Fig. 7. The same, X-ray diffraction pattern.—Fig. 8. *Sequoia sempervirens*, late wood. $\times 1,560$.—Fig. 9. The same, X-ray diffraction pattern.—Fig. 10. *Taxodium distichum* "Rotholz." $\times 1,040$.—Fig. 11. The same, X-ray diffraction pattern.



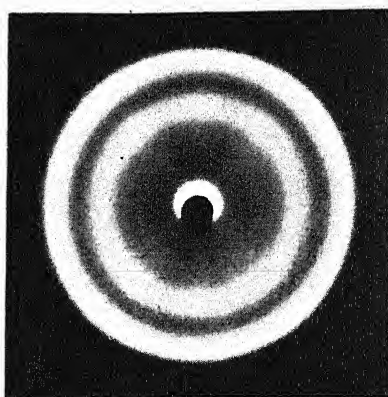
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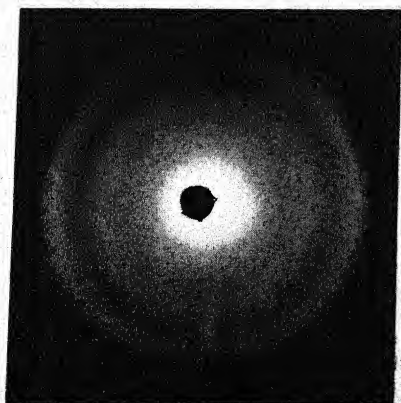
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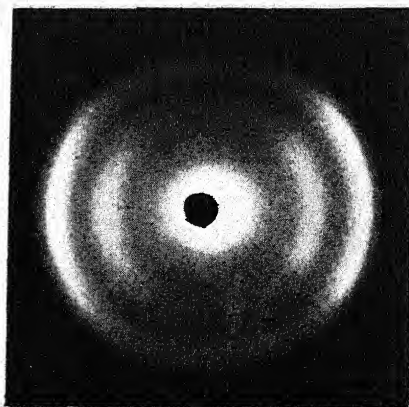
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morphological and histological variables in each specimen that is analyzed.

In the X-ray analyses reported here, small pieces of early wood and late wood were used, which had tangential surfaces of 4×6 millimeters and radial thicknesses of from 1 to 2.5 millimeters. These specimens were first treated successively with hot alcohol, benzene, ether, chloroform and acetone for the extraction of fats, waxes, terpenes, resins, etc. The specimens were subsequently delignified by alternating treatments with chlorine water and dilute alcoholic ammonium hydroxide.⁶ The use of the latter reagent at room temperature, instead of hot sodium sulphite, enables one to remove the bulk of the lignin from wood without macerating the tissue. Much care must be exercised in drying the delignified specimens to prevent undue warping and splitting. Undelignified specimens should be retained as controls, since the more conspicuous features of the diffraction patterns of cellulose frequently may be detected in spite of the amorphous fogging produced by lignin and other amorphous constituents.

In obtaining X-ray diffraction data from wood, it is desirable to compare the diffraction patterns obtained by passing the X-ray beam (a) parallel to the long axis of the wood rays, *viz.*, *radially*, (b) at right angles to the long axis of both the tracheids and the wood rays, *viz.*, *tangentially*, (c) at right angles to the long axis of the tracheids and at 45° to the long axis of the wood rays, *viz.*, *diagonally*, and (d) parallel to the long axis of the tracheids, *viz.*, *longitudinally*. Furthermore, in making accurate analyses, it is advisable to obtain micro-photometric readings from the original negatives, since such data frequently reveal intensity maxima which may be overlooked in a visual examination.

A detailed discussion of the data will be presented in a subsequent paper dealing with the more purely physical aspects of the problem. Thus, the corroborative microphotometric data, with one exception, are omitted from this paper and illustrations of diffraction patterns are reduced to an essential minimum.

DIFFRACTION PATTERNS OF LATE WOOD.—The conventional procedure in dealing with cotton, ramie and other fibers is to orient the cells with their long axis at right angles to the beam of X-rays. Figure 7 illustrates a diffraction pattern obtained by passing a beam of X-rays through the late wood of *Pinus longifolia* Roxb. at right angles to the long axis of the tracheids and parallel to the long axis of the wood rays. This diffraction pattern closely resembles that of ramie, which is considered to be indicative of a very uniform orientation of chain molecules approxi-

⁶ 150 cc. ammonium hydroxide, sp. gr. 0.90, to 850 cc. 95 per cent ethyl alcohol.

mately parallel or in a spiral of about 3° to the long axis of the cell. This particular sample of wood was selected for X-raying because the broad central layer of the secondary walls of the tracheids exhibits unusual regularity in the orientation of visible striations and of crystals (fig. 5) parallel to the long axis of the tracheids. Thus, there is a perfect correlation between the X-ray diffraction pattern and the fibrillar orientation of cellulose in the central layer of the secondary wall.

It is significant in this connection, however, that the fibrils of the tenuous inner and outer layers of the secondary wall are oriented either transversely (fig. 1) or in helices of relatively low pitch (fig. 2). Therefore, these layers are intensely birefringent in transverse sections of the wood (fig. 6), whereas the central layer exhibits no birefringence even in relatively thick sections. The fibrillar orientations of the inner and outer layers of the secondary wall obviously produce no detectable effects in the X-ray diffraction pattern. This is due to two sets of factors. In the first place, the fibrillar orientation of the inner and outer layers fluctuates considerably not only in different cells, but also in different parts of the same cell. In other words, the angle of inclination is not constant as in the central layer, but fluctuates between 60° and 90° . Therefore, the diffractions produced by the inner and outer layers are diffused over broad arcs rather than concentrated in localized interference spots. In the second place, recent work with cotton (Berkley, 1939) has demonstrated that the orientation of the cellulose in the tenuous primary wall of the cotton hair cannot be detected in X-ray patterns after the secondary wall attains any considerable thickness. The inner and outer layers of the secondary walls in the late wood of *Pinus longifolia* are too tenuous in comparison with the breadth of the central layer for both sets of diffractions to be detected simultaneously. For similar reasons, the walls of the wood rays produce no visible images.

Figure 9 illustrates a diffraction pattern obtained by passing a beam of X-rays through the late wood of *Sequoia sempervirens* Endl. at right angles to the long axis of the tracheids and parallel to that of the wood rays. This particular sample of wood was selected for X-ray analysis because it exhibits the following combination of visible structural characters. The inner and the outer layers of the secondary wall are unusually thick⁷ and the fibrils of these layers exhibit considerable uniformity of orientation at right

⁷ The apparent thickness of the layers in polarized light is deceptive. Accurate measurements of the average breadth of the layers must be made under high magnification with non-polarized light.

Fig. 12-17. X-ray diffraction patterns.—Fig. 12. *Sequoia sempervirens*, late wood, X-ray beam perpendicular to the long axis of both the tracheids and the wood rays, *viz.*, tangentially oriented.—Fig. 13. The same, X-ray beam perpendicular to the long axis of the tracheids and at 45° to the long axis of the wood rays, *viz.*, diagonally oriented.—Fig. 14. *Picea rubra*, "compression wood," X-ray beam perpendicular to the long axis of the tracheids and parallel to the long axis of the wood rays, *viz.*, radially oriented.—Fig. 15. *Pinus ponderosa*, "compression wood," X-ray beam radially oriented.—Fig. 16. *Trochodendron aralioides*, early wood, X-ray beam radially oriented.—Fig. 17. *Sequoia sempervirens*, early wood, X-ray beam radially oriented.

angles to the long axis of the tracheids. The fibrillar orientation of the central layer is helical, the pitch fluctuating within relatively narrow limits, *viz.*, between 30° and 40° . Therefore, all three layers of the secondary wall exhibit anisotropy in transverse sections of the wood (fig. 8), but the inner and outer layers are more intensely birefringent than the central layer.

In the X-ray pattern (fig. 9) there are four pairs of conspicuous diffraction maxima in both the 002 and 101 diffraction rings. The long axes of the tracheids were arranged vertical to the page; therefore, reading clockwise from the top of the pattern, the pairs of maxima may be observed at approximately 0° and 180° , 52.5° and 232.5° , 90° and 270° , 127.5° and 307.5° , each pair presumably representing a set of fibrils or crystallites in the cell wall. The two pairs of interference spots at 52.5° and 232.5° and at 127.5° and 307.5° are indicative of a helical orientation of chain molecules with a pitch of approximately 37.5° . The two pairs result from the same spiral but from opposite tangential walls of the tracheids. The somewhat less conspicuous maxima of both the 002 and 101 diffraction rings at 0° and 180° are indicative of a transverse orientation of chain molecules, *viz.*, approximately at right angles to the long axis of the tracheids. The diffraction maxima at 90° and 270° suggest a third orientation of chain molecules parallel to the long axis of the cells. The helical and transverse orientations of chain molecules obviously correlate closely with the visible fibrillar orientations of the central layer and of the inner and outer layers of the secondary walls of the tracheids, since they occur in X-ray diffraction patterns obtained by passing the X-ray beam perpendicular to the long axis of the tracheids but in the radial, the tangential, and diagonal directions to them (fig. 9, 12, and 13). On the contrary, there are no visible longitudinal orientations in the tracheids which may be correlated with the diffraction maxima at 90° and 270° . Furthermore, the form, arrangement, and wall structure of the ray cells is such that these diffraction maxima cannot be attributed to them.

For the solution of such a paradox attention must be devoted to a study of the effects of variations in the shapes of cells and the thickness of the different wall layers upon X-ray diffraction patterns. Most of the tracheids in the wood of *Sequoia sempervirens* are rectangular (fig. 8). The broad, flattened, tangential walls are oriented at right angles to the beam of X-rays, whereas the radial walls are oriented parallel to the beam when it is passed perpendicular to the long axis of the tracheids and parallel to the long axis of the wood rays. Similarly the tangential walls would be parallel and the radial walls perpendicular to the beam when it is passed in the tangential direction. Curved surfaces, the parts between the radial and tangential walls, are reduced to a minimum in this sample. Three of the four pairs of interference spots in figure 9 obviously are due to the diffraction of X-rays in passing through the tangential walls of the tracheids. The diffraction maxima at 90° and

270° on the other hand are apparently due to X-rays which pass through the radial walls of the tracheids and are diffracted by the transverse cellulose in the outer and inner layers of the secondary wall. These spots were more pronounced, as would be expected from the more uniform diffracting surface presented by the tangential walls (fig. 8) when the X-ray beam was passed perpendicular to the long axes of both the tracheids and the wood rays, *i.e.*, tangentially (fig. 12). Diffractions produced by the curved parts of the walls and by diagonally oriented radial walls tend to produce relatively broad arcs and thus to bridge the gaps lightly between the various sets of interference spots. That the diffraction phenomena in figure 9 may be accounted for without resorting to a hypothetical system of longitudinally oriented chain molecules can be demonstrated by the absence of the spots at 90° and 270° in figure 13, which was obtained by passing the beam of X-rays through the specimen perpendicular to the long axis but diagonal to the radial and tangential surfaces of the tracheids. If the interference spots had resulted from longitudinally arranged cellulose, they should not have disappeared in this pattern. Their disappearance in the diagonal pattern indicates that they were caused by a diffraction of the X-rays by the end-on position of the transverse cellulose in the outer and inner layers of the secondary wall.

DIFFRACTION PATTERNS OF "COMPRESSION WOOD."

—Figure 11 illustrates a diffraction pattern obtained by passing a beam of X-rays through the "compression wood" of *Taxodium distichum* (L.) Rich. at right angles to the long axis of the tracheids and parallel to that of the wood rays. As in figure 9, there are diffraction maxima indicative of helical and transverse orientations of chain molecules, but there are no detectable interference spots to indicate parallel arrangement. The tracheids of this particular specimen of "compression wood" are characterized by their large size, relatively thin ($3.5\ \mu$) secondary walls and somewhat compressed cylindrical form. The fibrillar orientation of the outer layer is dominantly transverse. As in other tracheids of the "Rotholz" type (Hartig, 1896), the inner layer is composed of radio-helical bands, having a pitch of 44° – 48° to the long axis of the tracheids. In this layer, the long axis of the fibrils is oriented parallel to that of the helical bands. Thus, both the inner and the outer layers are conspicuously birefringent in transverse sections of the wood (fig. 10). The isotropy or feeble birefringence of the intervening layer is due, not to a longitudinal orientation of fibrils, but to the fact that this layer is composed largely of non-cellulosic constituents.

The transverse cellulose of the outer layer of the secondary wall will account for the pair of interference spots at 0° and 180° of figure 11. The two pairs of interference spots at approximately 50° and 225° and at 132.5° and 310° can be ascribed to the radio-helical bands of the thick inner layer of the secondary wall. There are no maxima at 90° and 270° such as occur in figure 9. That the absence of

such maxima is correlated with the more cylindrical shape of the tracheids is indicated by the fact that other samples of "Rotholz" (figure 14) having cylindrical tracheids exhibit similar diffraction patterns regardless of variations in the thickness of the secondary wall and of its constituent layers.

Figure 15 illustrates a Debye-Scherrer type of diagram obtained from the "Rotholz" of a young stem of *Pinus ponderosa* Lawson. Such diffraction diagrams are commonly interpreted as resulting from random orientation of cellulose crystallites in the cell wall. It is significant in this connection, however, that the fibrillar orientation as indicated by visible striations, iodine crystals and optical and swelling anisotropy is dominantly transverse in the outer layer of the secondary wall of the tracheids and is prevalingly helical in the inner layer. Furthermore, the tracheids are very short and exhibit—both in radial and tangential views—a high ratio of pronounced curvatures and other deformations, particularly in their tapered upper and lower thirds. *A priori* one would expect the straight parts of the tracheids—i.e., those oriented parallel to the long axis of the stem—to produce diffraction maxima as in figures 11 and 14, whereas the curved and deformed parts would tend to produce a ring-type of diffraction pattern, since the fibrils are oriented at varied angles to the beam of X-rays. The microphotometric readings recorded in figure 18 demonstrate that maxima do occur at 0° and 180° , 45° and 225° , and 140° and 320° which are barely detectable visually in figure 15.

DIFFRACTION PATTERNS OF EARLY WOOD.—The fibrillar orientations in the thin-walled tracheids of early wood fluctuate considerably, not only in different cells, but also in different parts of the same cell, in different lamellae of the same wall layer, and in different parts of the same lamellae. The widest range of variability occurs in the radial walls of the tracheids and is correlated with the occurrence of bordered pits and numerous contacts with ray cells. In general, the fibrillar orientations tend to be steeper in the central layer than in the inner and outer layers and in tangential walls than in radial ones (Bailey and Vestal, 1937a). The thickness of the secondary wall and *pari passu* of the central layer varies considerably in different samples of early wood and between the first-formed and last-formed tracheids of the early wood. Furthermore, it is significant that not infrequently there is less regularity in the form of the tracheids in early wood than in late wood. Many of the large, thin-walled tracheids of the early wood are of hexagonal form. Therefore, there commonly is less uniformity in the orientation of radial walls parallel to the long axis of the rays in early wood than in normal late wood.

In view of such facts as these, one should expect early wood to produce broad arcs or rings of diffraction when X-rayed at right angles to the long axis of the tracheids. Figures 16 and 17 illustrate such diffraction patterns of the early wood of *Trochodendron aralioides* Zieb. & Zucc. and of *Sequoia semper-*

virens Endl. Owing to the tenuous character of the central layer in the tracheids of the early wood of *Trochodendron*, the diffractions produced by the steeper fibrillar orientations (0° — 45°) of this layer tend to blend into those produced by the lower fibrillar orientation (45° — 90°) of the inner and outer layers. On the contrary, in the case of the early wood of *Sequoia* where the central layer is thicker and has a higher ratio of fibrillar orientations between 20° and 45° , the broad arcs of diffractions produced by this layer may be differentiated from those due to the

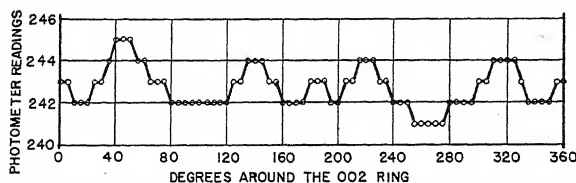


Fig. 18. Photometric readings from same film as figure 15.

fibrillar orientations (45° — 90°) of the inner and outer layers.

DISCUSSION.—There is a close correlation between the position of the long axis of the visible fibrils in the secondary walls of tracheids and that of the cellulose molecules in the crystallites as shown by the X-ray diffraction patterns that are produced from such walls. In other words, the evidence presented on the preceding pages supports the widely held view that to a large extent the long chain molecules and, therefore, the crystallites of cellulose are oriented within fibrils with their long axis parallel to the long axis of the fibrils. The X-ray data afford no support for Preston's assumption of a single spiral orientation of cellulose throughout the secondary wall of coniferous tracheids. On the contrary, they demonstrate that the orientation of the chain molecules fluctuates markedly in different layers of the secondary walls of coniferous and dicotyledonous tracheids.

With the exception of figure 7, the X-ray diffraction patterns taken perpendicular to the long axis of the tracheids are clearly indicative of more than one orientation of chain molecules and correlate closely with evidence obtained by the study of visible fibrillar orientations, of optical anisotropy, of swelling anisotropy and of predetermined planes of hydrolysis. In the case of the late wood of *Pinus longifolia*, the uniformly longitudinal orientation of fibrils in the broad central layer of the secondary wall correlates perfectly with the isotropy of this layer in transverse sections of the late wood (fig. 6) and with the X-ray diffraction pattern (fig. 7) produced by this layer. The inner and outer layers having fibrillar orientations of from 60° to 90° are intensely birefringent in transverse sections (fig. 6), but are so tenuous in comparison with the thickness of the central layer that they produce no conspicuous diffraction arcs in the X-ray photograph (fig. 7). If it be assumed from the X-ray diffraction pattern that there is a single longitudinal orientation of cellulose throughout the secondary wall, the inner and outer

layers in figure 6 should not exhibit birefringence. If one assumes that there is a single spiral orientation of cellulose throughout the secondary wall and that the appearance of layering is due to considerable angular dispersion of chain molecules within the central layer,⁸ this layer should exhibit some birefringence in transverse sections, and the X-ray diffraction pattern should not indicate such uniform longitudinal orientation of chain molecules.

It should be emphasized again in conclusion that, in selecting and preparing plant material for X-ray-ing and in interpreting X-ray diffraction patterns it is essential to be fully informed concerning the numerous morphological, histological, chemical and other variables in the material that is being analyzed. Where the layers of cell walls vary considerably in thickness, X-ray diffraction patterns may afford no evidence concerning the orientation of chain molecules in the more tenuous layers. Thus, X-ray diffraction patterns of mature cotton hairs give no clue concerning the orientation of cellulose in the primary wall. A ring-type of diffraction pattern is not indicative necessarily of a truly random orientation of chain molecules, but may be due (a) to deviations in the orientation of cellulose in the successively formed lamellae of cell walls, (b) to deviations of orientation in different cells of a particular specimen, (c) to curvatures and other deformations of the shape and orientation of cells within a specific specimen or (d) to a combination of such variables. Furthermore, certain variations in the type of diffraction pattern may be due to the shape and the arrangement of cells within a particular specimen, rather than to peculiarities of the orientation of cellulose within the walls of the cells.

Therefore, in dealing with the tracheids in wood, it is necessary to obtain reliable clues regarding the following variables:

1. The ratio of parenchymatous cells to tracheary tissue and the types of diffractions which they produce.
2. The shape and arrangement of the tracheids in transverse sections of the wood.
3. The shape and arrangement of the tracheids in radial and tangential longitudinal sections of the wood.
4. The comparative thickness of the wall layers.
5. The fibrillar orientations in the successively formed lamellae of these layers.

⁸This is contradictory, since the angularly dispersed chain molecules are not in alignment with a single spiral orientation. In Preston's (1939) revised diagram the alignment of chain molecules in surface view of the central layer does not correspond with the orientations figured in sectional views of this layer.

6. Fluctuations in the fibrillar orientations in different tracheids of the same specimen and in different surfaces of the same cells.

7. Morphological changes that may occur during chemical pretreatments and drying.

SUMMARY

The most reliable means at present of studying the fibrillar orientations of cellulose in individual layers of tracheids are microscopically visible striations and crystals that may be induced to form in the elongated porosities of the cellulosic matrix of unswollen walls. The over-all average or dominant arrangement of the crystalline cellulose may be best obtained from X-ray diffraction patterns.

Careful drying, delignification and maceration of wood produce minor changes in the orientation of fibrils but no pronounced deviations in orientation, such as from longitudinal to transverse or from right-handed to left-handed helices, etc.

There is a close correlation between fibrillar orientation, optical anisotropy, swelling anisotropy, predetermined planes of hydrolysis and X-ray diffraction patterns. This indicates that the chain molecules and crystallites of cellulose are oriented within fibrils with their long axis parallel to the long axis of the fibrils.

The fibrillar orientations of cellulose in the secondary walls of tracheids fluctuate markedly, not only in wood from different parts of the same tree but also in different layers of the same cell wall and frequently also in different lamellae of the same layer.

The fibrillar orientations of the inner and outer layers fluctuate between transverse and helices of comparative low pitch (45° – 90°), whereas those of the central layer usually deviate between longitudinal and helices of relatively steep pitch (0° – 45°). Uniform orientation of cellulose throughout the successive lamellae of a wall layer is exceptional rather than typical of tracheids.

The use of the polarizing microscope and of X-ray diffraction patterns alone may lead to serious misconceptions regarding the orientation of cellulose in cell walls, unless the numerous morphological, histological, chemical and other variables in plant materials are accurately visualized and accounted for.

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SPECIFICITY OF PYRIDOXINE FOR EXCISED TOMATO ROOTS¹

William J. Robbins

ROBBINS AND SCHMIDT (1939a, 1939b) reported that pyridoxine (vitamin B₆) beneficially affected the growth of excised tomato roots in a mineral-sugar solution containing thiamin. Bonner and Devirian (1939) found pyridoxine without effect upon the growth of excised roots of peas, radish and flax but obtained positive results with those of tomato. Bonner (1940) found no effect on excised roots of alfalfa, clover, or cotton but beneficial results with excised roots of *Datura stramonium*, sunflower, carrot and five strains of tomato. Stoutmeyer (1940) reported the rooting of the cuttings of some kinds of plants was improved by applications of pyridoxine, while others showed no response. Robbins (1941) found the excised roots of two inbred strains of tomato and their heterotic hybrid offspring to respond to pyridoxine in the presence of thiamin. White (1940) concluded that pyridoxine was of no benefit to the growth of excised tomato roots. Day (1941) found pyridoxine beneficial to the growth of excised tomato roots in an agar medium containing thiamin.

The present paper is not intended to account for the failure of White to obtain the beneficial effects of pyridoxine on excised tomato roots observed by Robbins and Schmidt, by Bonner and Devirian, by Bonner, by Robbins, and by Day, but to report on the specificity of the compound. Many of the vitamins and vitamin-like growth substances show a high degree of specificity; a small change in molecular structure reduces their effectiveness or eliminates it entirely. Other work which will not be reviewed here has shown the specificity of thiamin, nicotinic acid, riboflavin and pantothenic acid. The effect of pyridoxine on excised tomato roots also is quite specific.

METHODS AND MATERIALS.—The strain of excised tomato roots employed in these experiments was that originally isolated by Robbins and Schmidt (1938). This strain has been in culture since September, 1935, and has been maintained by transfers at monthly intervals in a mineral-sugar solution containing thiamin since October, 1936, and in a mineral-sugar solution containing the thiazole² inter-

mediate of thiamin since April, 1937. The numerous successive passages in a solution limited to mineral salts, sugar and thiamin on the one hand or to mineral salts, sugar and thiazole on the other insure that the roots used as inoculum in the experiments reported here contained no growth substances not synthesized by the roots other than thiamin or its derivatives.

The excised tomato roots were grown individually in 125 ml. Erlenmeyer flasks each containing 50 ml. of a modified Pfeffer's solution³ and either 1 per cent or 2 per cent cane sugar as indicated. The roots were incubated at 25°C. in diffuse light.

For inoculation, a root which was three or four weeks old was fragmented by a razor blade into pieces about a centimeter long. One fragment was transferred to each flask in which the effect of pyridoxine or an analogue was to be observed. Each fragment contained one or more growing points.

All glassware, as is customary in my laboratory, was thoroughly cleaned with chromic acid cleaning mixture or with a nitric-sulfuric acid cleaning mixture and thoroughly rinsed with tap water and distilled water. All chemicals were of chemically pure grade. The pyridoxine and thiamin were Merck's synthetic preparations.

Through the courtesy of Merck and Co. the following analogues of pyridoxine were obtained. For convenient reference the identification number⁴ furnished by Merck and Co., as well as the name of each compound is given:

- 39RD1421 Hydrochloride of triacetate of vitamin B₆.
- 39RD1422 Hydrochloride of diacetate of vitamin B₆.
- 39RD1442 Hydrochloride of B-methyl ether of vitamin B₆.
- 39RD1443 Hydrochloride of 2, 4, 5 trimethyl-3-hydroxypyridine.
- 39RD1029 2-methyl-3-amino-4-hydroxymethyl-5-amino-methyl pyridine dihydrochloride.
- 39RD1030 2-methyl-3-hydroxy-4-ethoxymethyl-5-hydroxymethyl pyridine hydrochloride.

³ This solution contained per liter 0.333 g. Ca(NO₃)₂, 0.083 g. MgSO₄·7H₂O, 0.083 g. KNO₃, 0.042 g. KCl, 0.083 g. KH₂PO₄ and 0.0025 g. Fe₂(SO₄)₃. In addition the following trace elements were added in ppm.: 0.05 B, 0.05 Mn, 0.005 Zn, 0.002 Cu and 0.005 Mo.

⁴ For brevity the portion of these numbers, 39RD or 41RD, is omitted later in referring to these compounds; for example, compound 39RD1421 is called #1421.

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² This is 4-methyl-5-β-hydroxyethylthiazole. It is referred to in this paper as thiazole.

- 39RD1031 2-methyl-3-amino-4-ethoxymethyl-5-amino-methyl pyridine dihydrochloride.
 39RD1032 2-methyl-3-hydroxy-4, 5-epoxydimethyl pyridine hydrochloride.
 39RD1797 Hydrochloride of 2, 4-dimethyl-3-hydroxy-5-hydroxymethyl pyridine.
 41RD3882 2-ethyl-3-hydroxy-4, 5-bis-(hydroxymethyl)-pyridine hydrochloride.
 39RD510 Lactone of 2-methyl-3-amino-4-hydroxymethyl-5-carboxypyridine.
 39RD511 Lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine.

Each of these compounds was added in amounts of 1, 10 and 50 millemicromoles ($m\mu$ moles) per flask in the presence of 10 $m\mu$ moles of thiamin. The growth of the excised tomato roots in solutions supplemented with the pyridoxine analogues was compared with their growth in solutions supplemented with molecularly equivalent amounts of pyridoxine. Five replications of each treatment were usually used, but in some instances ten replications were employed. Observations were made at intervals during the course of each experiment, and at the end of about two months the roots were dried at 100°C. and weighed.

EXPERIMENTAL RESULTS.—The diacetate and triacetate of pyridoxine (#1421 and #1422) were as effective as pyridoxine. Roots which had grown for twenty-eight successive passages in a mineral-sugar solution containing thiazole were used for inoculum. The Pfeffer's solution contained 2 per cent sugar, and the roots were grown from October 12, 1939, to December 14, 1939 (table 1). The pyridoxine analogues in this experiment had been added to the nutrient solutions before sterilization in the autoclave, and it was thought that the heating might have caused decomposition with the formation of free pyridoxine. The experiment was repeated, but the diacetate and triacetate were filtered sterile and added to the nutrient solution after it had been sterilized with heat. In this instance also the acetates

were as effective as pyridoxine. Not only was the amount of growth obtained with the acetates equal to that secured with molecularly equivalent quantities of pyridoxine, but the appearance of the roots was the same. The hooks and curls previously described (Robbins and Schmidt, 1939b) in solutions containing thiamin and pyridoxine were observed also in the solutions containing the acetates.

Compound #1442 differs from pyridoxine in having a methoxy group in place of the hydroxyl group in the third position on the pyridine ring (table 4). It was not effective as a substitute for pyridoxine (table 1). Because of the possible benefit from 1 $m\mu$ mole of compound #1442 (table 1), the experiment was repeated with additions of 1, 2, 10 and 50 $m\mu$ moles of the compound per flask. These proved ineffective.

Compound #1443 differs from pyridoxine in having the hydroxymethyl groups in the fourth and fifth positions replaced by the methyl radical. This substance did not replace pyridoxine for excised tomato roots (table 1).

Similar results were obtained with compounds #510, #511, #1029, #1030, #1031, and #1032 (table 2). In this experiment fragments of roots which had grown for thirty-one passages in Pfeffer's solution supplemented with thiamin were used for inoculum, and the experiment ran from June 23, 1939, to August 24, 1939. Of these #510, #511, #1029 and #1032 had neither a marked beneficial nor detrimental effect up to 50 $m\mu$ moles per flask;⁵ #1031 inhibited growth somewhat where 50 $m\mu$ moles were added per flask, and #1030 was decidedly toxic even when 10 $m\mu$ moles were present (table 2). The last compound is of particular interest because it differs from pyridoxine only in having an ethoxymethyl group in the fourth position on the

⁵ It is doubtful whether the slightly greater growth obtained with 1 $m\mu$ of compounds #510, #511 and #1029 is significant.

TABLE 1. Dry weights of excised tomato roots grown in presence of pyridoxine or some of its analogues in Pfeffer's solution containing cane sugar and thiamin. Period of growth, two months.

Additions to 50 ml. of Pfeffer's solution plus 2% cane sugar and 10 $m\mu$ moles thiamin	No. roots weighed	Ave. dry wt. per root mg.	Range dry wts. mg.
None	4	8.9	7.5-11.5
1 $m\mu$ mole pyridoxine	5	17.0	13.9-20.5
10 $m\mu$ moles pyridoxine	5	24.9	18.2-29.9
50 $m\mu$ moles pyridoxine	5	21.5	17.5-26.4
1 $m\mu$ mole #1421	5	19.9	12.5-29.0
10 $m\mu$ moles #1421	5	17.0	9.6-20.5
50 $m\mu$ moles #1421	5	30.8	22.2-42.6
1 $m\mu$ mole #1422	4	17.0	13.0-20.6
10 $m\mu$ moles #1422	5	27.9	7.2-40.8
50 $m\mu$ moles #1422	5	32.1	18.7-40.3
1 $m\mu$ mole #1442	5	14.6	8.6-34.1
10 $m\mu$ moles #1442	5	10.0	7.5-13.3
50 $m\mu$ moles #1442	5	8.8	3.8-12.6
1 $m\mu$ mole #1443	5	7.0	4.1- 9.7
10 $m\mu$ moles #1443	5	11.2	8.0-19.2
50 $m\mu$ moles #1443	5	9.4	8.0-12.6

TABLE 2. Dry weights of excised tomato roots grown in the presence of pyridoxine or some of its analogues in Pfeffer's solution containing cane sugar and thiamin. Period of growth, two months.

Additions to 50 ml. of Pfeffer's solution plus 2% cane sugar and 10 mμ moles thiamin	No. roots weighed	Ave. dry wt. per root mg.	Range dry wts. mg.
None	5	5.5	1.8- 7.6
1 mμ mole pyridoxine	5	7.3	6.1- 9.0
10 mμ moles pyridoxine	5	15.2	12.0-20.7
50 mμ moles pyridoxine	5	30.2	24.9-36.6
1 mμ mole #510	5	9.0	3.9-12.0
10 mμ moles #510	4	7.4	3.4-10.5
50 mμ moles #510	5	6.4	5.6- 8.2
1 mμ mole #511	5	9.2	6.9-12.8
10 mμ moles #511	5	8.3	5.9-10.9
50 mμ moles #511	5	3.2	2.3- 5.1
1 mμ mole #1029	5	9.5	2.5-18.5
10 mμ moles #1029	5	7.1	3.2- 9.6
50 mμ moles #1029	5	6.6	2.1- 9.5
1 mμ mole #1030	5	6.9	3.1-10.4
10 mμ moles #1030	5	1.2	0.1- 3.3
50 mμ moles #1030	5	0.2	0.1- 0.3
1 mμ mole #1031	5	7.5	5.7-10.3
10 mμ moles #1031	5	5.0	0.6- 7.5
50 mμ moles #1031	5	2.6	0.1- 4.5
1 mμ mole #1032	5	6.8	5.3- 8.5
10 mμ moles #1032	5	8.0	5.2-10.3
50 mμ moles #1032	5	5.3	3.6-10.2

pyridine ring instead of a hydroxymethyl radical (table 4).

Compounds #510 and #511 were used in a second experiment with 1, 10 and 50 μg. per flask in the presence of 10 μg. of thiamin. The inoculum was from roots which had grown for twenty-eight passages in a solution supplemented with thiamin. Neither benefit nor injury was observed with either compound.

Compound #1797 which differs from pyridoxine in having a methyl instead of a methoxy radical in the fourth position was not found to replace pyridoxine. It was used both filtered sterile and autoclaved. Up to 50 mμ moles per flask it was neither beneficial nor detrimental.

Compound #3882 differs from pyridoxine by having an ethyl instead of methyl radical in the second position on the pyridine ring. Pfeffer's solution containing 1 per cent cane sugar instead of the 2 per cent used in the other experiments reported in this

paper was used with this compound. It was an effective substitute for pyridoxine (table 3); in fact 1 mμ mole per flask was more effective than an equivalent amount of pyridoxine. In the experiment reported in table 3, fragments of roots which had grown for forty-nine successive passages in Pfeffer's solution supplemented with thiazole were used as inoculum, and the experiment ran from June 11, 1941, to August 7, 1941.

The experiment was repeated using as inoculum fragments of roots which had grown for fifty-five successive passages in Pfeffer's solution supplemented with thiamin. The roots were grown from July 5, 1941, to September 4, 1941, and the results were similar to those of the first experiment.

The chemical structure and results obtained with excised tomato roots as well as those reported by Unna (1940) for rats, are summarized in table 4.

DISCUSSION.—In general the results obtained on the effect of the pyridoxine analogues on excised to-

TABLE 3. Dry weights of excised tomato roots grown in the presence of pyridoxine or one of its analogues in Pfeffer's solution containing cane sugar and thiamin. Period of growth, two months.

Additions to 50 ml. of Pfeffer's solution plus 1% cane sugar and 10 mμ moles thiamin	No. roots weighed	Ave. dry wt. per root mg.	Range dry wts. mg.
None	5	5.9	3.6- 9.0
1 mμ mole of pyridoxine	10	7.4	3.1-10.2
10 mμ moles of pyridoxine	5	21.1	18.2-23.0
50 mμ moles of pyridoxine	5	22.7	20.6-24.9
1 mμ mole #3882	10	20.0	13.7-24.9
10 mμ moles #3882	10	19.5	12.0-23.3
50 mμ moles #3882	5	22.0	16.8-25.4

TABLE 4. Structure of pyridoxine and various analogues together with their activity on tomato roots and rats.

$ \begin{array}{c} \text{C-CH}_2\text{OH} \\ \diagup \quad \diagdown \\ \text{OH-C3} \quad \text{5C-CH}_2\text{OH} \\ \diagdown \quad \diagup \\ \text{CH}_3\text{-C2} \quad \text{6C-H} \\ \diagup \quad \diagdown \\ \text{N} \\ \text{HCl} \end{array} $							
Compound	2	3	Position 4	5	6	Activity on excised to- mato root	Activity on rats after K. Unna
Pyridoxine . . .	CH ₃	OH	CH ₂ OH	CH ₂ OH	H	Active	Active
1421	CH ₃	CH ₃ COO	CH ₂ CH ₃ COO	CH ₂ CH ₃ COO	H	Active	Active
1422	CH ₃	OH	CH ₂ CH ₃ COO	CH ₂ CH ₃ COO	H	Active	Active
1442	CH ₃	CH ₃ O	CH ₂ OH	CH ₂ OH	H	Inactive	Slightly active 2%
1443	CH ₃	OH	CH ₃	CH ₃	H	Inactive	Inactive
1029	CH ₃	NH ₂ ·HCl	CH ₂ OH	CH ₂ NH ₂ ·HCl	H	Inactive	Inactive
1030	CH ₃	OH	CH ₂ OC ₂ H ₅	CH ₂ OH	H	Inactive	Partially active 10%
1031	CH ₃	NH ₂ ·HCl	CH ₂ OC ₂ H ₅	CH ₂ NH ₂ ·HCl	H	Inactive	Inactive
1797	CH ₃	OH	CH ₃	CH ₂ OH	H	Inactive	Inactive
3882	C ₂ H ₅	OH	CH ₂ OH	CH ₂ OH	H	Active	Slightly active— less than 2%
510	Lactone of 2-methyl-3-amino-4-hydroxymethyl-5-carboxy- pyridine					Inactive
511	Lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxy- pyridine					Inactive
1032	2-methyl-3-hydroxy-4, 5-epoxydimethylpyridine hydrochloride					Inactive	Slightly active— less than 2%

mato roots agree with their effects on dermatitis of rats as reported by Unna (1940). He found the diacetate and triacetate as active on rats as pyridoxine. Bohonos, Hutchings and Peterson (1941) found the diacetate active for *Lactobacillus lactis*, but the triacetate was inactive. It is probable that the acetates dissociate in solution yielding free pyridoxine. Unna found compound #1442 slightly active (about 2 per cent the activity of pyridoxine), #1030 about 10 per cent as active and #1032 less than 2 per cent as active as pyridoxine.

Möller, Zinna, Jung and Moll (1939) reported compound #1797 to have about 2 per cent the activity of pyridoxine on *Streptobacterium plantarum* (*Bacterium acetylcholini*), though Unna found it to be inactive for rats. No activity of these substances was observed for excised tomato roots. However, a small activity such as 2 per cent or 10 per cent might not have been detected with excised tomato roots under my experimental conditions. For example, compound #1030 was toxic to excised tomato roots in amounts which would have been effective if it had one-tenth the activity of pyridoxine, and compounds #1442 and #1032, which showed 2 per cent or less of the activity of pyridoxine for rats, might have evidenced activity for tomato roots in amounts of 500 mμ moles. Of particular interest is the activity of compound #3882 which was as effective, or even more effective, for excised tomato roots than pyridoxine. Unna, as reported by Harris and Wilson (1941), found this compound to have on rats less than 2 per cent the activity of pyridoxine. It would

be of interest to determine its activity on other organisms.

It appears clear from the results obtained with excised tomato roots in these investigations and those obtained by others with rats, lactic acid bacteria and *Streptobacterium plantarum* that pyridoxine has a high degree of specificity. The results obtained with the effect of the various pyridine derivatives as compared with that of crystalline synthetic pyridoxine indicate that acetylation of pyridoxine did not reduce its activity for excised tomato roots. Methylation or ethylation of one of the hydroxymethyl groups, methylation of the phenolic hydroxyl groups or replacement of one or more hydroxymethyl groups by methyl or amino groups reduced the vitamin activity materially or destroyed it entirely.

SUMMARY

The effect of twelve analogues of pyridoxine on the growth of excised tomato roots in the presence of thiamin was studied. Acetylation of pyridoxine had no influence on its beneficial action and the substitution of an ethyl for the methyl group in the second position on the pyridine ring did not reduce the activity. The other nine analogues were inactive. Pyridoxine has a high degree of specificity for the growth of excised tomato roots.

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WOOD STRUCTURE OF THE NATIVE ONTARIO SPECIES OF JUNIPERUS¹

M. W. Bannan

JUNIPERUS is represented in Ontario by three species. *J. communis* var. *depressa* Pursh is widely distributed, *J. horizontalis* Moench has a more or less similar range but is usually less common, and *J. virginiana* L. is limited to certain areas in southern Ontario. As is well known, the three species are of diverse habits. *J. horizontalis* is creeping with most of the branches and stems flat on the ground, *J. communis* var. *depressa* generally forms large mats with the proximal portions of the branches or stems more or less prostrate and the distal parts semi-upright, and *J. virginiana* is arborescent.

MATERIAL.—Specimens of branch and stem wood of *J. communis* var. *depressa* were gathered from various localities in southern Ontario but principally from old pasture land near Brighton, stony soil in the Kawartha Lakes region, on rocks in the Parry Sound district, and from a sand dune area near Inverhuron on Lake Huron. The latter site also provided most of the *J. horizontalis* material which was kindly collected for the writer by Dr. Kathleen and Mr. Donald Hull. Supplementary material came from the Lake Superior area and from Bruce peninsula. Collections of *J. virginiana* were more complete and consisted of the following: stems, branches, and rooted branches of mature trees growing in a dune area on the shore of Lake Ontario south of Brighton; stems and roots of mature trees growing in shallow stony soil overlying limestone in the area east of Picton; small trees on a gravelly hillside, and branches and stems of mature trees growing in stony soil in an adjoining level area north of Trenton; and small trees and the branches, stems, and roots of mature trees in a clayey soil east of Beamsville in the Niagara peninsula.

Only stem and branch wood of *J. communis* var. *depressa* and *J. horizontalis* was examined, but in the case of *J. virginiana* the studies were extended to in-

clude such parts of the tree as indicated in figures 1-8. Here the term "stems small trees" denotes the inner wood 1-3 mm. from the pith at the base of the stems of young trees 0.5-1 m. tall. In the case of branches only those growing on mature trees were used, and both the inner wood 1-3 mm. from the pith and the outer wood on the upper and lower sides 1-3 cm. from the pith were sectioned. The term "stems large trees" refers to the wood in the periphery of old stems 2-4 dm. in diameter. All roots collected were lateral roots located at distances of 1.5-3 m. from the bole of mature trees. Both the inner wood 1-3 mm. from the center and the peripheral wood 1-3 cm. outward were studied.

The root habit of *J. virginiana* evidently varies with the environment. Bunker and Thomson (1938) recorded roots at a depth of 24.5 feet in the Oklahoma panhandle area. Small trees up to a meter tall excavated by the writer from deep gravelly soil had strong tap roots extending in some instances to depths of nearly a meter. Most of the larger trees from which root samples were taken were, however, growing in very shallow soil overlying limestone, and here the root systems were necessarily flat and plate-like. No deep roots were available for study. In the Abietineae these were found to differ materially in their structure from lateral roots near the surface (Bannan, 1941a). The data in figures 1-8 refer only to lateral roots in the top 1-2 dm. of soil.

The data in figures 1-8 are based upon a study of approximately one hundred specimens of *J. virginiana*. In each figure the maximum average (i.e., the average in the specimen with the largest elements) is shown by the upper broken line, the grand average for all specimens by the middle solid line, and the minimum average (in the specimen with the smallest elements) by the lower broken line. The descriptions which follow apply only to *J. virginiana* unless

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otherwise stated, but in no important details was the wood in the other two species materially different.

TRACHEID SIZE.—The average size of the tracheids in different parts of the tree is shown in figures 1 and 2. The values here presented were determined from the early-wood tracheids in the growth rings and indicate the external dimensions of the cells from one middle lamella to the next. The determinations

were made from transverse sections and included both wide and narrow cells as they appeared in these sections. Hence the values for the tangential width are somewhat less than would have been obtained had only the central widest part of each tracheid been measured from tangential sections.

The general trends in size of tracheids in different parts of the tree were similar to those noted in *Thuja occidentalis* (Bannan, 1941c). The size increased outward from the center in stems and branches, and was much greater in most lateral roots than in the aerial parts of the tree. In the case of roots, however, the tracheid dimensions varied with conditions under which the roots were growing. Lateral roots in more or less uniform soil had the largest cells, whereas in roots wedged between stones the tracheids were small and thick-walled with late wood sometimes constituting a large proportion of the growth rings. The wood in these roots was of fine texture and had a stemlike aspect.

TRACHEID PITTING.—The intertracheary bordered pits on the radial walls varied considerably in size in different parts of the tree. The largest pits were found in the early wood of the growth rings in lateral roots where the diameter ranged from 12–18 μ with the average about 15 μ . In the outer wood of old stems the pits were on the whole slightly smaller, and in the inner wood of branches and young stems the usual range was from 7–10 μ , the average 8–9 μ . In all parts of the tree the size tended to decrease from the early to the late wood in the growth rings.

The pits were generally uniseriate, but occasionally biseriate arrangements occurred in the early wood of the growth rings, especially in the outer wood of old stems and throughout lateral roots. In the latter case the pits were usually opposite, rarely alternate. Crassulae were variable and ranged in shape from straight to curved and in degree of development from distinct to absent.

Unlike *Thuja occidentalis*, the torus was well developed in most pits and was generally larger than the pit aperture. In lateral roots the margin of the torus was, as a rule, finely serrate (fig. 9), but in some cases was very irregular in outline with conspicuous bands or arms extending to the edge of the pit chamber. This irregularity was even more marked in branch and stem wood (fig. 10 and 11).

Tangential pitting occurred characteristically in the late-wood tracheids, rarely in the early wood. In the latter case it was usually associated with injury or some other abnormality in the wood.

The number of pits per crossing field between tracheids and ray cells was exceedingly variable, varying from cell to cell, with position in the growth ring, part of the tree, size and shape of the ray cell, and size of the tracheid. In general the number of pits decreased from the early to the late wood in the growth rings, was greater in roots than in stems or branches, and decreased from the inner to the outer wood in both aerial and subterranean parts of the tree (fig. 3). The range in the average number of pits between early-wood tracheids and ray parenchyma cells of

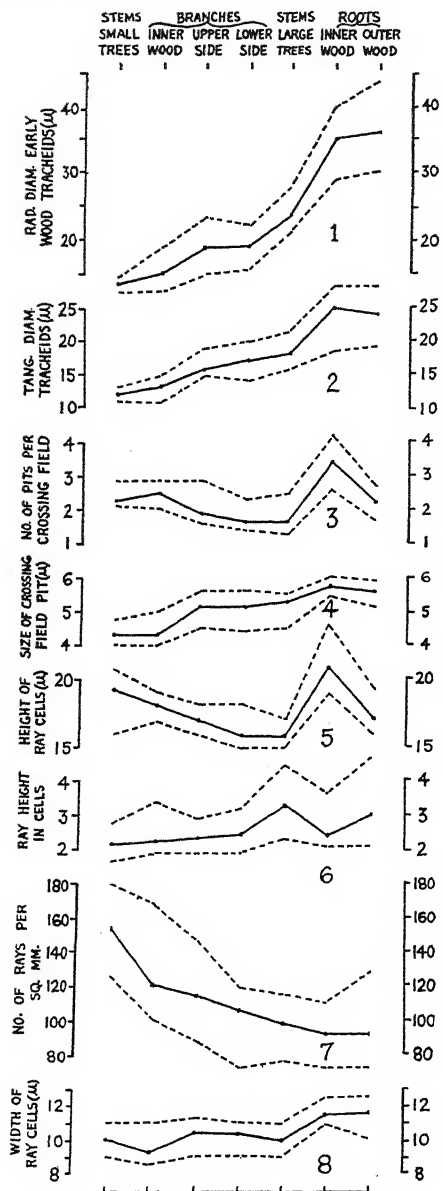


Fig. 1-8. *Juniperus virginiana*. Variation in different parts of the tree.—Fig. 1. Radial diameter of tracheids in early wood of growth rings.—Fig. 2. Tangential diameter of tracheids.—Fig. 3. Number of pits per crossing field between tracheids and ray parenchyma cells.—Fig. 4. Size of pits in crossing field.—Fig. 5. Height of ray parenchyma cells.—Fig. 6. Ray height in cells.—Fig. 7. Number of rays per sq. mm. of tangential section.—Fig. 8. Width of ray cells in tangential section.

normal radially elongated outline was from 3.4 in the inner wood of roots to 1.7 in the outer wood of old stems. The trends resembled those observed in *Thuja occidentalis* but the numerical values were about 50 per cent lower.

The diameter of the crossing-field pits also varied greatly, both within the same field and in different parts of the tree. On the whole the largest pits occurred in lateral roots and the smallest in the inner wood of branches and stems (fig. 4). As in *Thuja occidentalis* the size tended to increase outward from the center in stems and branches, and to decrease slightly outward from the center in roots. The average values were closely similar to those in that species.

The pit apertures in the crossing fields of the early-wood tracheids were usually slitlike with the inclination ranging from almost horizontal to steep. The size and outline of the aperture was variable and no definite trends were discovered in different parts of the tree.

XYLEM PARENCHYMA.—These cells ranged from plentiful to scarce. Their arrangement varied from widely scattered to compact tangential series, with tangential alignments predominating. No correlation was discovered between frequency of occurrence or distribution and position in the growth ring or location in the tree. The transverse walls varied from slightly thickened to very thick and strongly beaded. The thinner walled cells occurred most often in roots (fig. 12 and 13) and the thickest in stems (fig. 14–16), but there was much variation even within a single specimen.

SIZE AND DISTRIBUTION OF RAYS.—In general the trends in width, height, and distribution of rays in *J. virginiana* were similar to those recorded for *Thuja occidentalis* (Bannan, 1941c). Nearly all the rays were one cell wide, but a few, representing only a small fraction of one per cent, ranged from partially biseriate to multiseriate. The height varied from one to a maximum of thirty-two cells, the latter height being observed in the outer wood of an old stem. The average height varied greatly, both from specimen to specimen and in different parts of the tree. As in *Thuja* the greatest variation was noted in the periphery of old stems, where the range in average height was from 2.3 cells in one specimen to 4.5 cells in another with a grand average of 3.4 cells for all. The average height tended to increase from the inner to the outer wood in both stems and roots (fig. 6), and was slightly higher in roots than in stems or branches of similar size. The distribution of rays was also highly variable, but as a rule the rays were most numerous in the inner wood at the base of young stems and least frequent in the peripheral wood of old stems and throughout the wood of lateral roots (fig. 7). The rays in *J. virginiana* were noticeably more numerous than in *Thuja occidentalis*, the average distribution in different parts of the tree ranging from 91 to 155 rays per sq. mm. in the former as compared with a range of 53 to 87 in the latter.

The size and shape of the ray cells differed with position in the ray, location in the annual ring, and part of the tree. The cells in the first-formed parts of the rays were often vertically extended, whereas those in the succeeding parts were usually radially elongated. As a rule the latter cells were longer in the early wood of the growth rings than in the late wood. The height of these typical radially elongated ray cells tended to decrease from the inner wood toward the periphery and to be at a maximum in roots (fig. 5). The tangential width of the cells was also greater in roots than in other parts of the tree (fig. 8).

As stated above most of the rays were uniseriate throughout, but exceptional ones became multiseriate. Stages in the widening of such a ray are illustrated in figures 17–23. The ray began at the pith (fig. 17) and in the first few growth rings resembled ordinary rays in width and in size and shape of the ray cells (fig. 18). In the tenth ring, however, while the ray was still uniseriate, the cells became noticeably wider (fig. 19), and this increased width was maintained until the twenty-first ring (fig. 20). Here the ray widened to two cells and continued biseriate until the thirty-second ring (fig. 21–23), after which there was rapid expansion. A short distance farther out the ray connected with an adventitious root.

Sometimes the development differed in certain respects from that described above. Although the majority of the unusual rays originated in the vicinity of the pith, a few began in the secondary xylem. In some cases there was enlargement of the cells while the ray was still uniseriate, whereas in other instances no appreciable alteration in size or shape could be detected, the ray resembling an ordinary uniseriate ray up to the point of widening. The actual broadening was sometimes gradual, extending through several growth rings, while in other cases it was so sudden that the ray seemed to flare out like a funnel. After having widened some of the rays connected with adventitious roots, some were abruptly succeeded by several smaller rays, and others gradually narrowed. For instance one ray broadened to several cells, then was followed by smaller rays two of which underwent the following changes: one widened, then narrowed, widened again, split into smaller rays, one of which widened and connected with an adventitious root; the other widened, subsequently narrowed, widened again, was succeeded by smaller rays, and one of these widened and in turn was broken into smaller units.

As in *Thuja occidentalis* ray widening appeared to be due to subdivision of ray initials and gradual narrowing to failure of some of the initials to continue normal tangential division. Where the wide rays were abruptly succeeded by uniseriate rays the appearance in transverse sections was generally suggestive of a subdivision of the ray by penetration of fusiform elements between the ray initials. At times, however, some of the uniseriate rays were separated from the preceding wide ray by gaps equal to the width of a few tracheids. Whether the ray initials

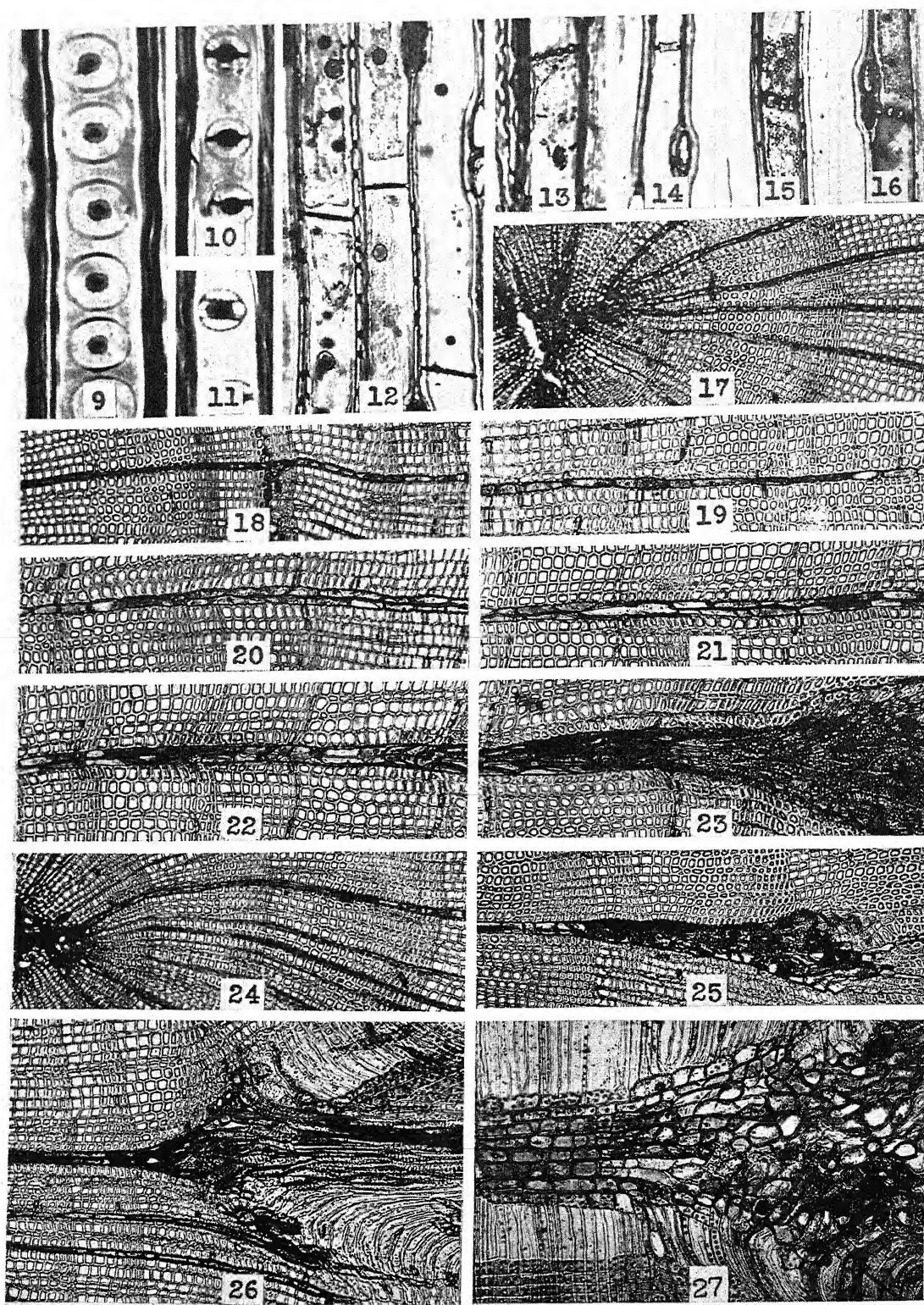


Fig. 9-27.—Fig. 9-23. *Juniperus virginiana*.—Fig. 9. Radial section of root wood showing shape of the torus.—Fig. 10, 11. Torus in old stem wood.—Fig. 12, 13. Tangential sections of root wood showing transverse wall of xylem parenchyma cells.—Fig. 14-16. Transverse walls of xylem parenchyma cells in old stem wood.—Fig. 17-23. Transverse sections of branch wood illustrating stages in development of unusual ray.—Fig. 24-27. *J. communis* var. *depressa*.—Fig. 24-26.

here involved were of recent origin or were old initials that had merely ceased normal division for a short time was not determined.

These unusual rays were very rare. None were discovered in old stem wood. In more than twenty small branches 0.5–1 cm. in diameter, collected from several sites, the average occurrence was only one in every 10 sq. cm. of tangential area. In the outer wood of old branches 2–5 cm. in diameter the average was one per 2.3 sq. cm., and in the wood of small trees 0.5 to 1 m. tall it was one ray per 1.7 sq. cm. The distribution was exceedingly erratic. For instance, two-thirds of the unusual rays noted in the thirteen old branches examined were in one specimen. The rays were sporadic in branches collected in the Niagara peninsula, but none were observed in six specimens cut from trees growing in the dune area near Brighton. Similarly in the case of the small trees three times as many rays were found in the Niagara material as in that obtained near Trenton in eastern Ontario.

RAY STRUCTURE.—Ray tracheids occurred only rarely in the various stems, branches, and roots examined. They were sporadic in both the ordinary uniseriate rays and in the unusual rays described above. When present in the ordinary rays they were located in or near the first-formed part where the ray was usually only a single cell high, although sometimes such new rays coalesced with older rays nearby and formed marginal rows along their upper or lower side. The number of ray tracheids in each new ray was never great, generally only a cell or two. The proportion of new rays containing tracheary cells was highly variable, ranging from a high percentage in some cases to very low in others. Counts were made of the number of ray rows containing ray tracheids and in most cases the proportion of such rows was less than 1 per cent of the total number of the rows in all the rays (new and old). The range was from 3 per cent in one specimen to nearly zero in most. No outstanding differences were noted between roots, stems, or branches.

The occurrence of ray tracheids in the unusual rays was also sporadic. The majority of these rays were parenchymatous throughout, but occasionally ray tracheids were interspersed among the parenchyma cells, both in the uniseriate and wide portions of the ray. When in the narrow parts, the ray tracheids were generally rectangular in shape and smooth-walled; whereas those in the wide portions were more varied in shape, and the walls sometimes had reticulate thickenings on the inside. When these rays connected with adventitious roots, gradations were observed from such reticulate cells to primary xylem tracheids in the newly formed root. As a rule tracheary cells were less frequent in the unusual rays in stem and branch wood of *J. virginiana* than in the comparable rays in *Thuja occidentalis*. Irregularity in cell shape, both of the parenchymatous and tra-

cheary cells, was also less marked. On the whole there was closer resemblance between the unusual rays in branch wood of *Juniperus* and root wood of *Thuja* than between the rays in branch wood of the two genera.

The structure of the parenchyma cells in typical uniseriate rays, especially that of the tangential walls, has been frequently described. Because the tangential wall is thicker and, hence, the recessions in it are deeper than in most Cupressineae, the thin areas have long been known as "juniperoid pits." Bailey and Faull (1934), however, state that these are not true pits but are deeply depressed pit-fields in a thickened primary wall. In material examined by the writer the tangential walls varied greatly, but on the whole the most prominently thickened or beaded walls occurred in the branches and the thinnest in the inner wood of lateral roots. In the latter the tangential walls ranged from strongly to only sparsely or delicately knobbed, and on rare occasions were nearly smooth. The horizontal walls were also variable, the combined thickness of the walls of the two adjoining ray cells ranging from 1.5–3 μ , with the thinner walls occurring most often in roots and the thicker most often in branches. As a rule the pit-fields were comparatively widely spaced but sometimes were approximate. Indentures, or recessions, in the horizontal wall adjoining the vertical wall were usually prominent, as Peirce (1937) has stated, but in occasional cells were absent.

Parenchyma cells in the unusual rays showed a greater range in structure, particularly in the wide portions of these rays. Gradations were observed from cells with thick heavily lignified walls on all sides to those with uniformly thin unlignified or only slightly lignified walls. Some of the cells were living, others dead, the proportion of the latter tending to increase as the ray widened.

RAYS AND ADVENTITIOUS ROOT FORMATION.—A formation of adventitious roots was observed in all three species of *Juniperus*. The freest development was noted in *J. communis* var. *depressa* and *J. horizontalis* where roots were formed along the lower sides of the decumbent branches. In the case of *J. virginiana* there was less opportunity for such rooting to take place owing to the arborescent habit of the species, and to the nature of the sites in which the trees usually grew. Most of the trees in the areas visited had lost their lower branches, but, where these were still living and were in contact with the ground, conditions did not favor rooting. The soil was usually stony with sod between the stones, so there was little chance for loose earth to collect around the branches, and the natural deposition of organic litter under the trees never seemed heavy enough to bury any of the branches. It was only in dune areas, where the lower parts of the bole and the proximal portions of the lowermost branches were covered by the shifting sand, that root development was ob-

Transverse sections of branch wood showing steps in development of unusual ray and its connection with an adventitious root.—Fig. 27. Radial section of branch wood showing structure of ray at point of connection with adventitious root. Fig. 9–11 $\times 640$, Fig. 12–16 $\times 310$, Fig. 17–27 $\times 105$.

served. Here branches which had been buried for some time produced many adventitious roots (fig. 30), and in some cases these evidently made a considerable contribution to the growth of the branches, for the latter were much larger in and above the rooted areas than in the proximal portions between the roots and the stem. For instance in the branch illustrated in figure 30 the cross-sectional area just above the rooted portion was approximately 3 times that of the proximal part adjoining the stem. The branches were usually bowed, turning from a more or less horizontal direction near the stem to an upright

In *J. communis* var. *depressa*, as in *J. virginiana*, all the adventitious roots examined connected with xylem rays. This relationship is illustrated in figures 24-26. The ray began at the pith (fig. 24) and in the inner two growth rings was uniseriate and composed exclusively of parenchyma cells resembling those in ordinary rays. In the third and fourth rings the ray cells widened somewhat, and in the fifth ring the ray became biseriate. Further broadening took place in the next three rings (fig. 25), after which the ray was succeeded by smaller units, one of which widened again in the thirteenth and fourteenth rings where a

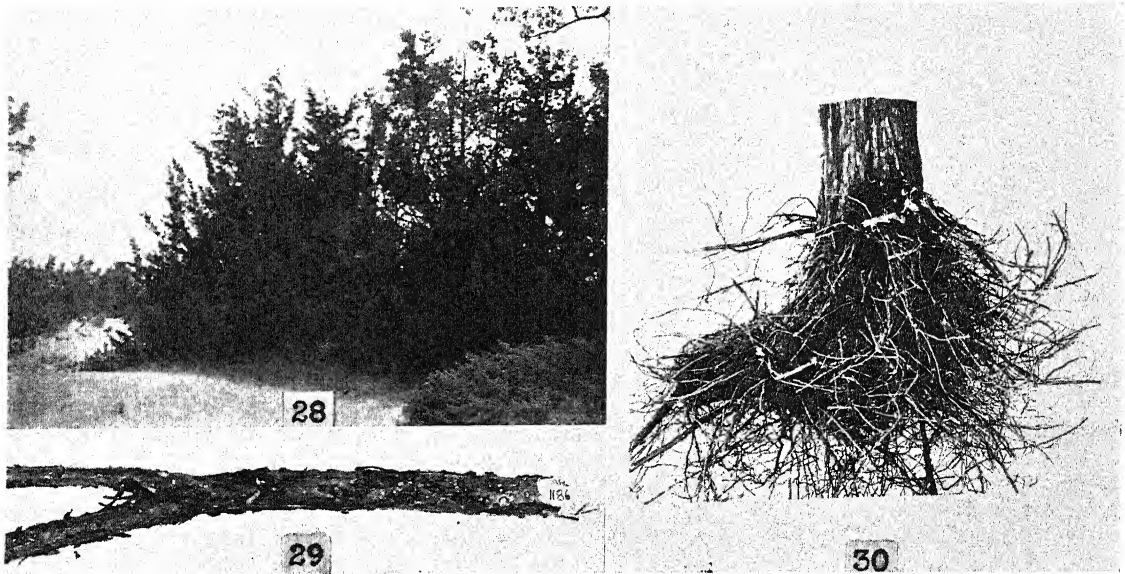


Fig. 28-30.—Fig. 28. Dune area showing in center a tree of *J. virginiana* whose lower branches have rooted and turned upright, and in the lower right *J. communis* var. *depressa*.—Fig. 29. Portion of branch of *J. communis* var. *depressa* showing partially formed roots.—Fig. 30. Buried portion of branch of *J. virginiana* showing profuse development of adventitious roots.

position farther out. Because of this upright habit and of the increase in growth which sometimes made the branches almost as large as the stem, the branches together with the stem could easily be mistaken at a distance for a group of separate trees (fig. 28).

In all the specimens of *J. virginiana* studied the adventitious roots connected with rays in the xylem. Seven of the rays were traced to their point of origin, and it was found that six began at the pith and one in the early wood of the second growth ring. All seven rays were initially uniseriate and resembled other rays in the xylem, but later were distinguished by gradual or abrupt broadening, sometimes preceded or accompanied by enlargement of certain of the ray cells as described above in the account on ray structure. In some instances the root was formed soon after the broadening had taken place, in other cases the ray repeatedly widened and narrowed or was succeeded by smaller rays before the final broadening and ultimate root formation were accomplished.

root was initiated (fig. 26). Some thirty-nine rays associated with root development were studied, and of these twenty-nine began at the pith and the other ten at various points in the secondary xylem from the first to the third growth rings. All were uniseriate at first, but in their subsequent development they differed considerably. Some widened gradually, others abruptly. Some connected directly with roots after the first extensive widening; others split up into small rays, certain of which in turn widened and ultimately connected with roots. The locations in the wood at which ray widening and root formation took place were inconstant, even in the same piece of branch. Some of the rays were exclusively parenchymatous (fig. 27), but in other rays varying numbers of ray tracheids were interspersed among the parenchyma cells both in the uniseriate and wide portions of the rays. On the whole these tracheary cells were more frequent than in *J. virginiana*.

The unusual rays were observed in the upright aerial portions of the branches as well as in the

buried parts. They were never numerous but usually occurred more often than in *J. virginiana*, the average distribution in several specimens collected from various points in southern Ontario being 1.2 rays per sq. cm. of tangential area. Although the rays were found throughout the length of the branches, functioning roots were formed only where the branches were in contact with the ground or were covered by soil. In a few cases small protuberances appeared on the lower sides of the aerial parts of the branches (fig. 29), and sectioning revealed these to have a structure resembling the initial stages of root formation. They appeared to be roots which had developed only far enough to bulge the bark but not break through it. These roots, like the functioning roots, connected with rays in the xylem which were generally of the unusual type. A similar relationship was described for *Thuja occidentalis* (Bannan, 1941b). In *J. communis* var. *depressa* these partially formed roots were exceedingly sporadic, being completely lacking on some branches and quite numerous on others (fig. 29). For instance, on the lower side of one branch there were as many as six or seven to the sq. cm. at distances as much as 0.5 m. above the ground. The ground was in sod, and there was no evidence to indicate that the parts of the branch bearing the arrested roots had been covered by soil or litter within the last few years when the roots were initiated.

In *J. horizontalis* the creeping branches rooted freely and all the roots studied were associated with xylem rays similar to those described for the other two species. Twenty-four of the rays traced to their origin extended to the pith, whereas twenty-three began at various points in the secondary xylem from the first to the fourth growth rings.

DISCUSSION.—Many of the trends in anatomical variation in *Juniperus virginiana* resembled those observed in *Thuja occidentalis*. Similar tendencies were noted particularly in such quantitative characters as size of the tracheids, size of the intertracheary pits, size and number of pits per crossing field between tracheids and ray cells, height and distribution of rays, and size of ray cells. In some cases the actual numerical values for the average and range of variation were similar in the two genera; in other cases they differed only slightly, but in one or two characters, as, for instance, the frequency of the rays, the average values were markedly different. Even in the latter case, however, the variation in different specimens was such as to render of doubtful value the use of this character for diagnostic purposes.

Certain of the anatomical characters more often regarded as diagnostic also varied considerably, although in some cases trends could be detected in different parts of the tree. For example, the tangential walls of the ray cells long considered to be a diagnostic feature of *Juniperus* wood, were usually strongly thickened and knobbed, but in roots particularly were highly variable, ranging from thick in most cells to thin and only weakly or sparsely beaded in others, and were on the whole definitely thinner

than in branch wood. The horizontal walls of the ray cells were usually comparatively thick with rather widely spaced pit-fields, but here again there was much variation, especially in roots where the walls tended to be thinner than in branches. The transverse walls of the xylem parenchyma cells were likewise variable, with the thinner walls occurring most often in roots and the thicker in branches or old stems. In *Thuja occidentalis*, on the other hand, no such tendency was observed, the walls of the parenchyma cells were variable in all parts of the tree without being noticeably thinner or thicker on the average in any one part. Because of lack of information on the trend and range of structural variability in most of the Cupressineae, no attempt will be made at this time to select particular characters which might prove useful for the identification of *Juniperus* wood or its separation from that of other genera.

Unusual rays resembling those reported for *Thuja occidentalis* occurred in all three species of *Juniperus*. In the latter these rays were generally more infrequent than in *Thuja*, and they usually contained fewer interspersed tracheary cells, but the distribution and structure were much too variable in both genera to have diagnostic value.

In *Juniperus*, as in *Thuja*, adventitious roots arising from stem or branch wood connected with rays in the xylem which were almost always of the unusual type mentioned above. Whether such relationship is general for the Cupressineae cannot be stated. Preliminary investigations indicate that different relationships obtain in certain other conifers, notably the Abietineae. In the rooted branches of *Abies* and *Picea* examined to date the roots originated from hitherto dormant buds, and in the few available specimens of *Larix* the roots arose from dwarf (leaf-bearing) branches.

SUMMARY

Many of the trends in anatomical variation in different parts of the tree in *J. virginiana* resembled those observed in *Thuja occidentalis*. Similar tendencies were noted in such characters as size of the tracheids, size and distribution of the intertracheary pits, size and number of pits per crossing field, height and distribution of rays, and size of the ray cells.

Other features usually regarded as valuable for purposes of identification such as the thickness and character of the walls of the ray and xylem parenchyma cells also varied considerably. Because of such variability it is clear that the selection of characters which will ultimately prove diagnostic must be held in abeyance until there is more complete knowledge of the range of variation in related forms.

An expansion of certain of the rays to a multiserial condition, such as observed in *Thuja occidentalis*, was also noted in all three local species of *Juniperus*. All adventitious roots arising from stems or branches connected with rays in the xylem, nearly always of this unusual type.

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THE DISTRIBUTION AND PREPARATION OF CITRUS PEROXIDASE¹

W. B. Davis

SZENT-GYÖRGYI (1928), citing Onslow (1921), speaks of the orange, grapefruit and lemon as typical "peroxidase plants," but apparently failed to find active peroxidase in the juice of the citrus fruits mentioned. The flesh and peel of the citrus fruits do not darken quickly on injury as does the flesh of potatoes, apples and many other fruits. According to Huszák (1937), ascorbic acid has so commonly accompanied peroxidase that some workers suspect that it is a part of the peroxidase system. Citrus fruits are well known for their ascorbic acid content. Although orange juice slowly darkens on storage, no striking change in color is noted when it is exposed to the air a short time. Wilson (1928) has suggested that the Maillard reaction is responsible for this change, which, however, is possibly due to other causes. Orange residues accumulating in the manufacture of by-products sometimes turn black. Willmott and Wokes (1926) made qualitative tests on the peel of citrus fruits.

While the above brief literature survey indicates that peroxidase should be found in citrus fruits, apparently little work is available to show this is actually the case.

This paper was written to prove the existence, and point out the distribution, of peroxidase in some citrus fruits and to describe some of the attempts to make a crude preparation of this enzyme. The properties of citrus peroxidase will be described in another paper.

DISTRIBUTION OF PEROXIDASE.—A preparation of citrus peroxidase was needed for work dealing with citrus juices. Since the peel of those citrus fruits which have an appreciable layer of albedo gives a strong reaction with guaiacum and hydrogen peroxide, an attempt was made to isolate peroxidase from Navel orange peel, which is quite thick. A slight modification of the alkaline extraction method of Willstätter was tried with unsatisfactory results. It appeared from later determinations that extracts from orange peel have little power to catalyze the

oxidation of pyrogallol by hydrogen peroxide. This experience with the peel led to a systematic search of the different tissues of available citrus fruits to find where peroxidase was located. The quantitative method of Balls and Hale (1933) was used. In this method the quantity of hydrogen peroxide used in the oxidation of pyrogallol is measured by titration with standard thiosulfate. The catalysis by peroxidase takes place at 30°C. and at pH of 8.00. The peroxidase values obtained by this method differ from the values obtained by the method of Willstätter and Stoll because of the difference in pH and temperature between the two determinations. One important feature is that peroxidase may be estimated in material containing catalase. The peroxidase units (P.U.) given in this paper are the same as the units described by Balls and Hale (1933). Results are shown in table 1.

Large quantities of peroxidase were not found in the tissue of any of the citrus fruits previously examined, except in the outermost layer of the peel of grapefruit. Tangerines, which became available at this stage of the investigation, were examined and appeared to have a greater content of peroxidase, particularly in the seeds, than any other citrus fruit previously examined. A detailed examination of the seeds revealed a high peroxidase activity in the inner coat. But the end of the seed opposite to its point of attachment to the fruit, the chalazal end, where the inner seed coat is thickened, contains very little peroxidase. The inner coat of some tangerine seeds darkens when left in contact with the crushed pulp for twenty-four hours. The outer seed coat appears to have little peroxidase activity.

In addition to the examinations of fruits mentioned in table 1, others were made of a variant of grapefruit called "Seedy" and of green Valencia oranges.² The Seedy grapefruit is of interest, not only on account of its high peroxidase content in the inner seed coat (as high as 1,436 P.U. per kilo), but also on account of the larger number of seeds per fruit; the supply of these fruits is, however, limited. The inner seed coat (not including the thickened chalazal end)

² Thanks are due to Mr. A. D. Shamel for calling attention to the Seedy grapefruit, and to Mr. Brown on whose ranch the fruit was grown.

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The advice of Dr. A. K. Balls, of the Enzyme Research Laboratory of this Bureau, in this work, is gratefully acknowledged.

TABLE 1. *Distribution of peroxidase in tissues of citrus fruits.*

	Peroxidase units extracted per kilo of fresh tissue			
	Navel Orange	Marsh Grapefruit	Lemon	Tangerine
Fruit tissue:				
Albedo	5.5	13.8	2.8	**
Flavedo, inner	14.0	*	*	*
Flavedo, outer	31.3	350-414	191.5	*
Endocarp	1.7	14.6	4.6	*
Sediment from juice	1.8	0.7	*	*
Peel	*	*	*	58.8
Seed tissue:				
Whole seed	*	147.0	*	231.8
Seed coat, inner	*	440-1,100	*	1,100-2,500
Seed coat, outer	*	5.5	51.0	*
Cotyledons	*	27.6	*	55.2

** = No analysis made.

of the seeds of immature Valencia oranges ran 1,220 P.U. per kilo.

The approximate peroxidase content of different tissues varies considerably, perhaps according to the age, locality where grown, and storage conditions. The moisture content of the different tissues also varies. The separation of inner and outer flavedo is an arbitrary matter. By the outer flavedo is meant the thinnest layer that can be pared from the outside of the fruit. This tissue includes but a small proportion of the oil cells.

As may be seen from table 1, the localized portions of citrus fruits, particularly of their inner seed coats, have high peroxidase activity. With regard to their peroxidase activity, these citrus fruit tissues are high in comparison with malt sprouts and horseradish (550 and 403 peroxidase units per kilo, respectively, according to Balls and Hale [1933]), the same method being used for all determinations.

PREPARATIONS.—To determine the best of a few peroxidase extractants, about one gram of inner seed coats of tangerines, grapefruit and lemon were mixed together and ground in a mortar with ether to a fine powder. Aliquots of this powder were again ground with the different extractants shown in table 2. The centrifuged extract was used for quantitative determinations. The loss of peroxidase activity after storage of the extract at 4°C. for forty-eight hours

is also shown. While ammonium sulfate and sodium bicarbonate were better extractants than phosphate buffer at pH 8, the latter proved to be more useful because an acetone precipitate separates easily from it.

Various attempts were made to prepare peroxidase from the inner seed coats removed by hand. In one case 84 grams of inner coat of tangerine seeds testing 920 P.U. per kilo (a total of 77.28 units) were disintegrated in 300 ml. M/20 phosphate buffer at pH 8 in a Waring blender (Davis, 1939) at 3 to 4°C. Acetone was added to the liquid after it was squeezed through muslin by hand, until a precipitate occurred. The precipitate dried with ether weighed 1.334 grams. The activity was 18,400 P.U. per kilo of precipitate (a total of 24.55 units). The recovery was 29.34 per cent. In another case 33 grams of inner seed coat of lemon, testing 661 P.U. per kilo, were extracted with 2 per cent ammonium sulfate and the enzyme was precipitated by saturation of the extract with ammonium sulfate. The precipitate (1.5 grams) had 6,700 units per kilo, or a total of 10.0 units. This was 45.85 per cent of the original 21.8 units. A preparation of high activity has not yet been obtained from grapefruit flavedo.

Since only approximately 5 to 7 per cent of the seed of citrus fruits is inner coat, the accumulation of a large quantity of this material is a difficult matter.

TABLE 2. *Relative effectiveness of various extractants for removing peroxidase from inner seed coats.*

Extractant	Peroxidase units extracted per kilo of fresh tissue	Loss of activity after 48 hours storage at 4°C.
		Per cent
Distilled water	783	4
M/20 phosphate buffer, pH 9	1,207	6
M/20 phosphate buffer, pH 8	1,643	7
2 per cent sodium bicarbonate	1,765	13
2 per cent ammonium sulphate	1,835	13

Some method of getting sufficient quantities for large-scale operation was sought. While tangerine seed was not available in quantity, lemon seed was secured in twenty-five pound lots.² The separation of peroxidase from the ground mixture, consisting of both seed coats, is difficult on account of the slimy substance (apparently pectin) in the outer coat which makes up the bulk of the mixture. Therefore, the inner coat was largely separated from the outer by the following process. First, most of the cotyledons were removed by crushing the dry seed between rollers and screening. Then the inner coat was loosened from the outer in a ball mill, and the lighter inner coat particles were separated from the outer by again screening in a current of air from a fan. The bran thus separated and containing a small proportion of outer coat and cotyledons was extracted with petroleum ether to remove oil. In this way, 600 grams of bran was prepared.

Two hundred and fifty grams of this bran, containing 47.8 units of peroxidase, were extracted with 2 per cent ammonium sulfate at 4°C. for about twenty

² The cooperation of Mr. H. W. Hall of the Lemon Products Company of the California Fruit Growers Exchange in separating these seeds is acknowledged.

hours in the cold. The filtered extract was saturated with ammonium sulfate, and again filtered. In the precipitate 20.55 units were recovered, thus giving a recovery of approximately 43 per cent. However, the preparation contained only 736 peroxidase units per kilo of precipitate, requiring further purification to reach the activity of preparations made from hand-removed inner coat.

SUMMARY

The distribution of peroxidase in different tissues of orange, grapefruit, lemon and tangerine is described, and the high activity in the inner seed coat of these fruits, especially tangerines, is pointed out. Hand-removed seed coats gave the highest activity in crude preparations. That it may be possible to make peroxidase on a large scale is indicated from experiments with lemon seeds, which are available in quantity.

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SOME FEATURES OF THE STRUCTURE OF ARCTOSTAPHYLOS VISCIDA¹

Bernice E. Doyel

ARCTOSTAPHYLOS is a genus of Ericaceae including erect, evergreen shrubs, some of which attain a height of twelve feet or more. The branches are crooked with a red, glabrous bark. The alternate leaves, which vary greatly in size, are generally elliptical and are often sharply mucronate. For the most part they are glabrous and grey-green, although the degree of color differs with the species; many are recurved. The scaly axillary buds begin to unfold late in spring (statements here given as to the seasons or months at which stages are reached apply to the species in southwestern North America; not necessarily to those which are not). These buds quickly give rise to stems a few centimeters long and bearing perhaps a dozen leaves. Most of these annual shoots terminate in panicles which remain dormant until fall; the form of the resting panicle is characteristic of the species (Weislander and Schreiber, 1939). The flower buds,

in the axils of bracts and embraced between pairs of bractlets, are recognizable late in spring. After the first rain of the season the flowers start to swell; the pedicels, beginning to elongate, carry them beyond the protection of the bracts and bractlets. The blooming season is generally from December until February. The pedicels of the flowers and fruits of some species are covered with minute, glandular hairs. Because of the presence of these hairs the inflorescence is sticky to the touch. The flowers range from white to light pink in color; they are hermaphroditic and ordinarily pentamerous. The sepals are separate, recurved at the tips, and finely ciliate. The petals are united to form a urceolate corolla; the tips of the petals curve outward. The stamens are twice the number of petals and are attached to the bases of the petals. Each anther bears a pair of recurved appendages and dehisces through two pores. The filaments are ribbon-like with expanded bases; the bases

¹ Received for publication November 26, 1941.

are hairy, the upper portions smooth. The stamens in the planes of the petals are definitely longer than those in the planes of the sepals. The base of the ovary is surrounded by a glandular disk. All parts of the pistil are glabrous in some species; the ovary is glandular in others. The lobed stigma extends above the corolla in the open flower. In many cases the stigma and style continue to be attached after the ovary has developed into a young fruit. The ovary is superior and is divided into from four to ten cavities. Each cavity contains one epitropous ovule. The red fruit, smooth or glandular, is subglobular and depressed at both ends. The seeds are enclosed in nutlets which may be separate, or a number or all of them may be united into one.

The characters as given exclude from this genus several segregates (*Comarostaphylos* Zuccarini; *Arctous* Niedenzu; *Xylococcus* Nuttall; *Ornithostaphylos* Small; and *Schizococcus* Eastwood; cf. Small (1914) and Eastwood (1934a)). Thus limited, the genus includes fifty-three species, according to the latest monograph (Eastwood, 1934b), to which may be added *A. cratericola* Donn. Sm., making fifty-four.

The distribution is circumpolar with an extension southward in western North America to Central America; the greatest development of species is in California where it makes up a large percentage of the abundant chaparral.

The observations presented in this paper were made upon *Arctostaphylos viscida* Parry (1887). This species is distinguished by shrubby stature, grey leaves, a slender and recurved nascent inflorescence, the cauline part of the panicle (rachis and pedicels) glandular, ovary (and fruit) glabrous. Parry gives no definite type locality; the species has been found in the foothills of the Sierra Nevada from Tulare County northward to Jackson and Josephine Counties, Oregon, and thence southward in the coast range to Lake County.

The material used was collected at Mormon Island in El Dorado County by Dr. Herbert F. Copeland. Collections were made at intervals of two weeks during the 1939-1940 season of development and again in the spring of 1941. Bouin's fluid was used for killing and fixing the earlier material; in the later collections FAA was also employed. All the slides were stained with a triple combination of safranin, light green, and aniline blue.

It is with gratitude and appreciation that I acknowledge the generous help and unfailing interest extended to me by Dr. Copeland. I am also indebted to him for figures 1, 2, 10, and 11.

VASCULAR ANATOMY OF THE FLOWER (fig. 5).—The vascular supply in stem, rachis of panicle, and pedicel of *Arctostaphylos* is arranged in a typical siphonostele. After the vascular tissue enters the receptacle of the flower it breaks up into a number of whorls of bundles which continue up into the flower and supply its various parts. The first whorl of bundles to be given off are those five which supply the sepals. Alternating with, and above the sepal bun-

dles, is a whorl of five petal bundles. The vascular supply of the ten stamens is in two whorls of five bundles each. Those supplying the stamens which are in the planes of the petals are the first to be given off. Slightly above these, and in the planes of the sepals, the second whorl departs from the cylinder. The numerical symmetry of the bundles of the flowers, and therewith of the gross members of the flowers, ceases at this point. The next whorl of bundles above the stamen bundles is that of the carpel dorsals. The level of these is only slightly above that of the petaled stamen bundles, but they are not in the planes of any of the previous vascular bundles. There were eight carpel dorsal bundles in the flower used for the study of the vascular anatomy; this number is not the same in all flowers. Some distance above the preceding whorl of carpel dorsal bundles, the vascular cylinder breaks up into some fourteen bundles, half of which, alternating (approximately) with the carpel dorsals are placentals, one to each ovule, while the rest enter the style and fade out.

Gaps are formed in the vascular cylinder where the bundles supplying the sepals, most of the petals, and the carpel dorsals are given off. There are none above the stamen bundles and there was none above one of the petal bundles in the flower used for this study. The longitudinal extent of each of the gaps is small, and at no time do they overlap to give the cylinder a dissected appearance.

THE STAMEN.—The first appearance of the stamen is late in July when the buds are still less than one millimeter in length. At this time the anther alone is discernible. During the ensuing two months the filament grows slightly; early in October it begins to elongate rapidly. The filaments of the five stamens opposite the petals are slightly longer than those of the five stamens in the planes of the sepals. The dilated base of each filament is covered with a rather heavy growth of simple, unicellular hairs which are more conspicuous on the dorsal surface than on the ventral.

The anther of the juvenile stamen stands erect. There is a short ridge on the outer side of the lower or proximal end of each lobe (fig. 7). This ridge is to become a part of the margin of the pore. The appendages, of which each anther bears two, are attached to the bases of the lobes, near the lower ends of the ridges. As the filament elongates (October to December) it becomes bent inward at the summit until at maturity the original ventral surface of the anther is on the outside and is flush with the filament. Thus the filament of the mature stamen enters the upper end of the anther and the distal part of the stamen bundle, descending within the anther, is parallel with the proximal part ascending the filament. Meanwhile, the appendages have grown so as to extend horizontally outward from the upper ends of the anthers.

The four microsporangia of each anther are differentiated in September. Each microsporangium consists of a body of pollen mother cells covered by a tapetum of uninucleate cells and two or three layers

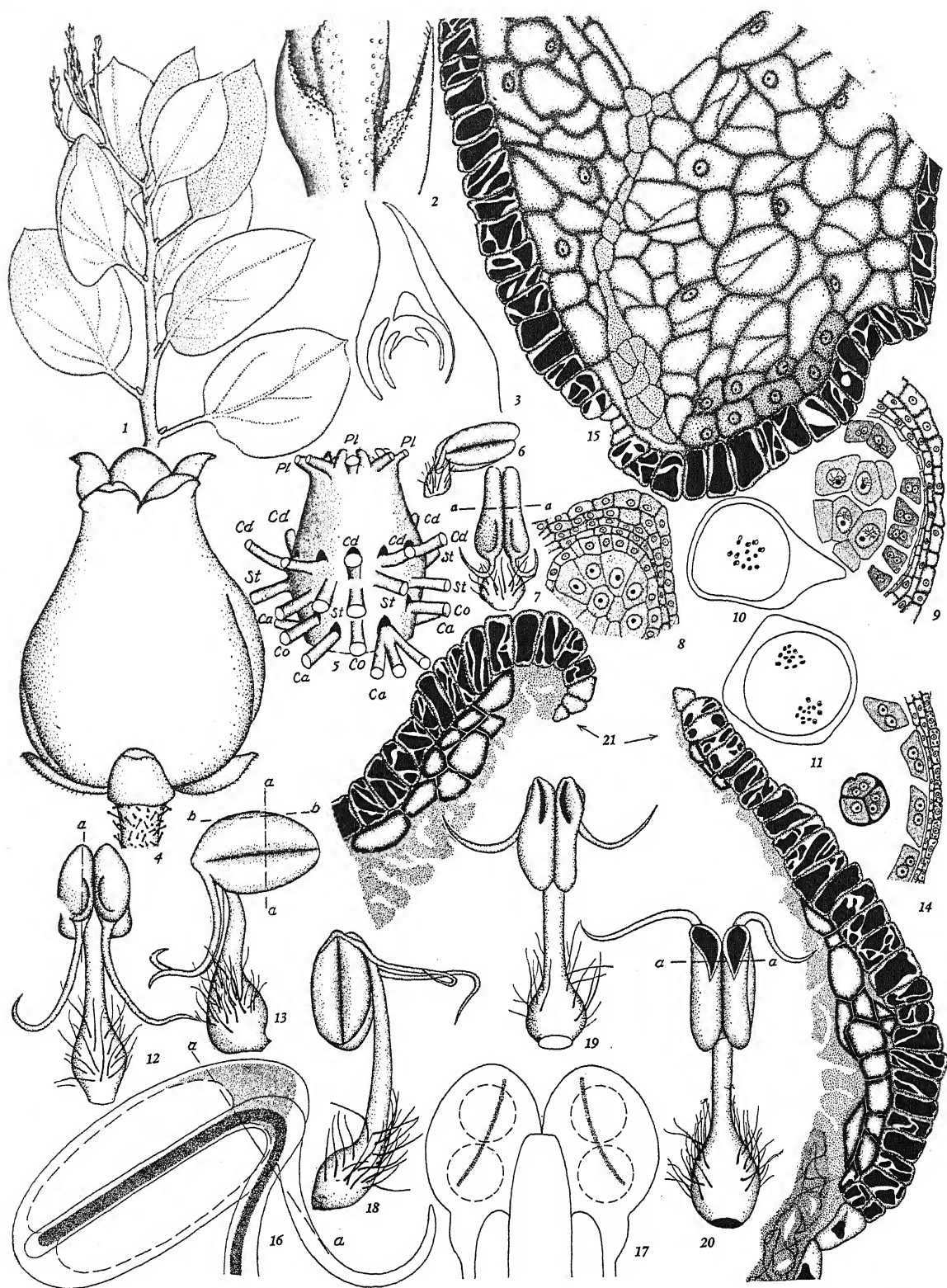


Fig. 1-21.—Fig. 1. Branch showing leaves and young flower buds. $\times 4/5$.—Fig. 2. Portion of inflorescence. $\times 8$.—Fig. 3. Diagram of the flower apex in June. $\times 40$.—Fig. 4. Flower. $\times 8$.—Fig. 5. Model of the vascular system in the receptacle of the flower, $\times 40$; Ca, sepal bundle; Co, petal bundle; St, stamen bundle; Cd, carpel dorsal; Pl, placental bundle.—Fig. 6, 7. Young stamens. $\times 12$.—Fig. 8. Area marked a-a in fig. 7, $\times 320$.—Fig. 9. Cross section of stamen showing

of wall cells and an epidermis (in which tannin subsequently accumulates) (fig. 8). The microspore mother cells enter the prophase of reduction division early in October. In the meantime the cells of the tapetum have become binucleate (fig. 9, 14). Reduction division occurred in December in 1939; iron-acetocarmine preparations made at that time revealed the haploid number of chromosomes to be thirteen (fig. 10, 11). Between reduction division and the maturing of the pollen grains, the tapetum disintegrates. The mature pollen grains, found almost immediately after reduction division, are binucleate and are in tetrads (fig. 14).

Frontal sections in the region of the external ridge, of an anther fixed in January, show a line of cells in the first stage of collapse, extending from the proximal end of each microsporangium almost to the epidermis at the base of the ridge on the side away from the median plane of the anther. In the part of the epidermis beyond the end of this line, the cells are smaller and contain no tannin (fig. 15). Sagittal sections of stamens of the same age reveal the collapsed cells to be in a plate (fig. 16). The plate extends across the two pollen sacs with each lobe; it is separated from the epidermis by one row of cells. As the anther matures the width of the row of collapsed cells increases, until at maturity all the cells between the microsporangia and the epidermis have disintegrated. At approximately the same time the wall between the dorsal and ventral microsporangia of each lobe breaks down.

Coincident with the appearance of collapsed cells in the interior, certain hypodermal cells assume distinctive characters. A cross section of the anther near the pores shows these as a parabola or incomplete ellipse, laterally on both sides and ventrally, but not dorsally, with regard to each lobe of the anther. The cytoplasm of these cells remains relatively dense after the cytoplasm of the adjoining cells has been reduced to a narrow peripheral layer. With the collapse and subsequent disappearance of the cells in the area of the pore, the cytoplasm of these cells becomes restricted to a layer adjacent to the cell wall; the nuclei disintegrate and a secondary wall with simple pit-pairs is deposited (fig. 21). The structure described appears to be a rudimentary endothecium; its function, if any, is to strengthen the outer side of the pore.

Soon after the anther becomes inverted and before the flower has opened, the epidermal cells at the proximal end contract. This causes a slit to form among the cells without tannin at the outer base of the ridge. The width of the opening increases, so as to form a pore, by the curling under of the edges. The pores reach full opening as the flowers open (fig. 20).

EMBRYOGENY.—The epitropous ovules first appear in July at which time they are nothing more

than knobs. In December, when the tapetal cells of the anther have become binucleate, the ovule is found to contain an archesporial cell covered by a single layer of nucellar cells, these being covered in turn by an integument, several cells thick and extending to the end of the nucellus (fig. 23). As in all Ericales that have been studied, the archesporial cell functions as the megaspore mother cell. After the megaspore mother cell appears, it develops no further until the integument has grown beyond the end of the nucellus to form the micropyle. The outer layer of epidermis becomes tanniferous as development proceeds. Two successive divisions of the megaspore mother cell then occur to form a tetrad of spores (fig. 24). The chalazal megaspore is functional; the remaining three disintegrate and eventually disappear. The nucleus of the functional megaspore undergoes the usual three successive divisions which result in the formation of a normal embryo sac (fig. 26). The egg and synergids are rather small. The polar nuclei unite before fertilization. The three antipodals soon disintegrate. At this stage in the development the base of the funiculus begins to become extended across the micropyle to form an obturator. Following fertilization the endosperm nucleus divides twice; each nuclear division is followed by cytokinesis. At the same time the micropyle becomes obliterated by growth of the surrounding cells. The second and third cells of the row of four which form after the division of the endosperm nucleus divide in all planes to form the endosperm. The chalazal cell of the four divides longitudinally to form two cells (fig. 28). These later unite into one (fig. 29), and eventually this cell becomes the chalazal haustorium. The course of development in the micropylar cell of the four endosperm cells was not adequately determined. It gives rise to the micropylar haustorium, and there is evidence of a degeneration and destruction of the nearest cells of the endosperm proper under the influence of this structure. It was impossible to distinguish cells or nuclei in the haustoria; in each case they were vacuolated masses of cytoplasm (fig. 31, 32).

The zygote undergoes no divisions until after the endosperm has become many celled. Early in February it elongates to form a tube in which the nucleus is located at the end toward the chalaza (fig. 29). A series of transverse divisions forms a multicellular uniseriate suspensor. The course of the suspensor in the haustorium can be traced, although no cell walls or nuclei can be distinguished (fig. 30, 31). Eventually the terminal cell divides obliquely; continued divisions build up a globular embryo. After the globular embryo develops, the suspensor is absorbed so that the embryo has no contact with the micropylar haustorium (fig. 32).

The stony layer surrounding each seed begins to develop in February. As it becomes thicker and

microspore mother cells. $\times 320$.—Fig. 10, 11. Pollen grains. $\times 920$.—Fig. 12, 13. Stamens. $\times 13$.—Fig. 14. Area marked a-a in fig. 13. $\times 320$.—Fig. 15. Area marked b-b in fig. 13. $\times 320$.—Fig. 16. Area marked a-a in fig. 12. $\times 80$.—Fig. 17. Diagram of anther across the pollen sacs and showing the course of the collapsed tissue. $\times 80$.—Fig. 18, 19, 20. Mature stamens. $\times 12$.—Fig. 21. Cross section of stamen showing collapsed cells and the endothecium. $\times 320$.

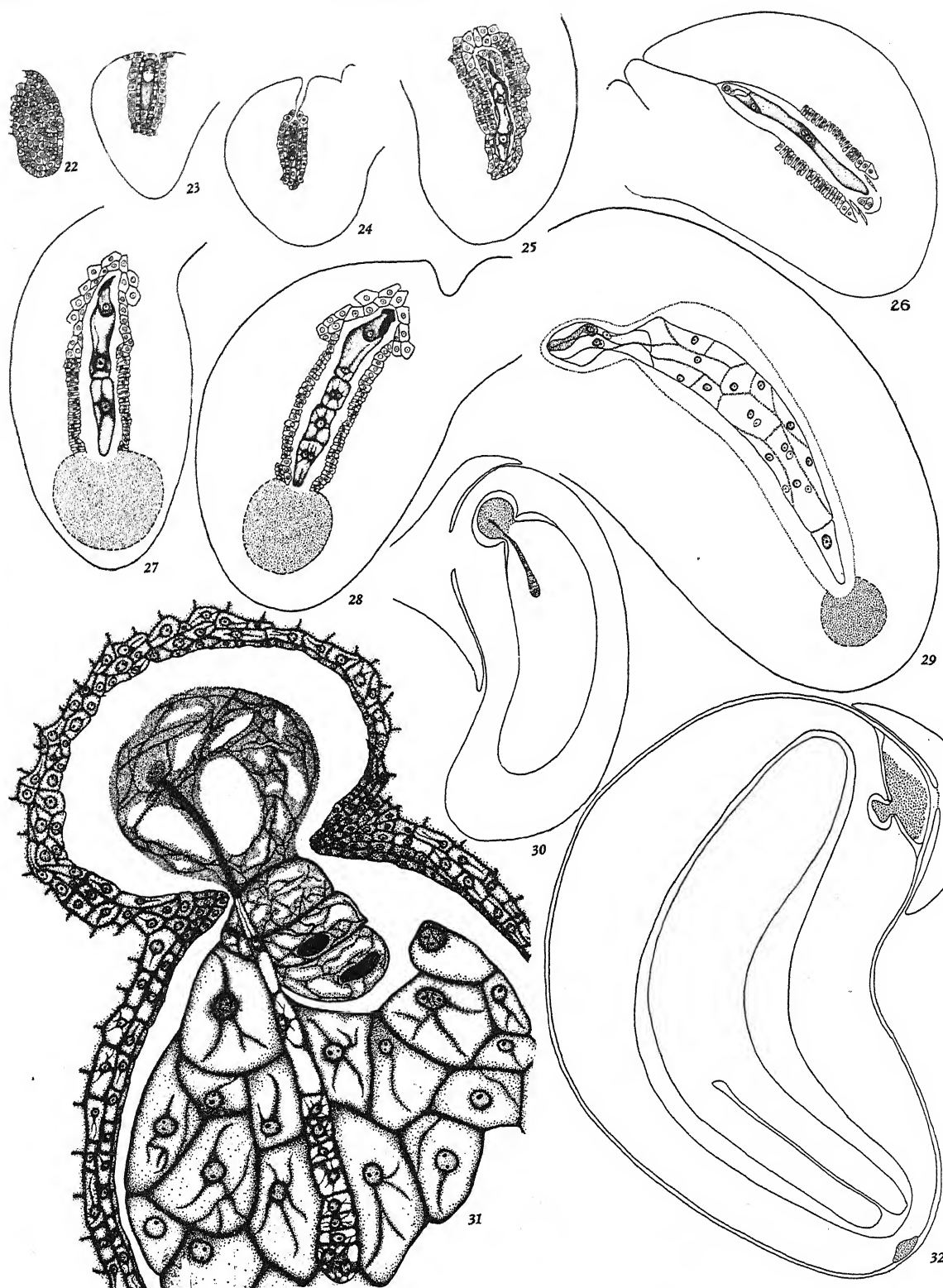


Fig. 22-32. Longitudinal sections of ovules and young seeds in successive stages of development.—Fig. 22-29, 31, $\times 160$.—Fig. 30, 32, $\times 40$.

harder it creates difficulty in sectioning the seed and determining its structure. In June it was found that the embryo had become a cylinder nearly as long as the endosperm, divided to somewhat less than half its length into two cotyledons. The plane of division may either be anticlinal or periclinal to the median plane of the flower. The radicle is bent toward the axis of the fruit. It has grown forward so as to point not toward the micropylar haustorium but past it, on the side toward the summit of the fruit. Both haustoria by this time are much compressed and stain darkly, as if moribund. The obturator has developed so as to form an external cap over the micropylar end of the seed. It is perhaps to be interpreted as a reduced placenta. Such is apparently the mature structure of the seed; seeds collected late in July show no further development.

DISCUSSION.—The features of the structure of *Arctostaphylos* as set forth in this paper agree in all essentials with those given by Samuelsson (1913) and Schnarf (1931) for the embryogeny and with those set forth by Matthews and Knox (1926) for the structure of the stamen.

It is hoped that the data presented will be found useful in the study of the evolution of the Arbutoidae and of their place in the phylogeny of the Ericales, when other members of the group have been examined.

SUMMARY

Arctostaphylos viscida is a shrub common in the chaparral of northern California.

The course of the vascular bundles in the receptacle is described.

The young stamen is erect and bears two appendages at the base. As the stamen matures the filament bends. Thus, the apparently distal end of the mature stamen is really the proximal end. Each lobe of the young anther consists of an epidermis (in which tannin develops), a hypodermal layer (in which a scant rudimentary endothecium develops at the morphologically proximal end), one or two additional layers of wall cells, a tapetum which has a binucleate stage, and pollen mother cells. The haploid number of chromosomes is thirteen. The mature pollen grains are in tetrads. Early in the ontogeny of the stamen, a plate of collapsed cells develops in each lobe of the anther between the pollen sacs and the morphologically proximal end where the pores are to form. By the time the pores open, the area of collapsed cells has increased, so that all the cells between the individual microsporangia and all the cells between the microsporangia and the epidermis are included. Contraction of the epidermal cells in this region causes the pores to open.

The ovules are epitropous. In each a single integument consisting of several layers of cells covers the nucellus. The latter is of one layer of cells. The archesporial cell functions as the megaspore mother cell. The embryo sac is of the normal type. A chalazal and a micropylar haustorium are produced. The embryo is borne on a suspensor.

The plane which divides the embryo into its two cotyledons may be either anticlinal or periclinal in relation to the median plane of the flower.

The mature seed is surrounded by a thick layer composed of stone cells.

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A DEVELOPMENTAL STUDY OF THREE SAPROPHYTIC CHYTRIDS.

III. SEPTOCHYTRIUM VARIABILE BERDAN¹

Helen Berenice Berdan

THE DISCOVERY, isolation from a single spore germinated on agar, and the culture of this chytrid have been described in a preliminary report. The generic and specific analyses were also given at that time (Berdan, 1939). Although no essentially different features have been observed since then, cultures of the fungus have been grown in cellophane and lens paper. On cellophane, early stages of zoospore germination and thallus formation showed so clearly that it was considered desirable to add these details, as well as others concerning the later life history, to the previous record.

THE ZOOSPORE AND THALLUS DEVELOPMENT.—The living zoospore (fig. 1, 2) is hyaline, spherical to oval in shape, 4–6 μ in diameter, and with a single posterior flagellum 30–40 μ in length. Normally one highly refractive globule 0.7–3.0 μ (usually about 2 μ) is present in the lightly granular and more or less vacuolated cytoplasm. A small, very shiny granule is situated at the insertion of the flagellum. The method of swimming is typical of most chytrids and the active period varies from a few minutes to several hours, depending upon conditions. The zoospores may undergo the amoeboid, creeping, rolling and jerking movements described many times for the spores of various chytrids. From the sporangia of one heavily infected piece of substratum hundreds of thousands of zoospores emerge. Large, single sporangia have been estimated to discharge from 25,000 to 75,000 spores. Consequently, many variations and irregularities are observed among them, although the percentage of such malformations cannot be computed for comparison with other chytrids. Often, actively swimming spores lack refractive globules, while others, apparently perfectly formed except for a flagellum, are not able to swim at all. Because it is impossible to concentrate attention for a long period on any one spore among the seething numbers milling about on a slide, it has not been ascertained that non-motile spores remain amoeboid and capable of producing infection, as Zopf (1884) has claimed for *Amoebochytrium rhizidioides*. Frequently, spores may contain several globules or bear several flagella. When strong light falls upon the sporangia, immature ones may shed partially developed spores. Then imperfectly-shaped doubled and tripled spores are found and many contain a large, single vacuole causing the spore to appear as an empty ring to which the flagellum is attached. The refractive globule should be measured only from spores of mature sporangia whose discharge has been normal, as otherwise the variability exceeds the normal range. Two or three flagella may be given off from a single point in the spore, the flagellum may be unusually short,

and the loops mentioned for *Cladochytrium hyalinum* (Berdan, 1941a) and *Catenochytridium carolinianum* (Berdan, 1941b) are just as common in this genus (fig. 3). The nucleus appears as a dense area in the cytoplasm. Large spores discharged under unusual conditions may have two or more nuclei present.

Germination of the zoospore is essentially similar to that described for *Entophlyctis* (Karling, 1931a), *Endochytrium* (Karling, 1937a; Hillegas, 1940), etc., of the Rhizidiaceae, and *Cladochytrium replicatum* (Karling, 1931b, 1935) and *C. hyalinum* (Berdan, 1941a) of the *Cladochytriaceae*, but numerous variations may occur. Many zoospores swell and degenerate after coming to rest. The germinating ones usually develop a solitary germ tube, although several may be produced. The tube penetrates into the substratum, where it branches and soon produces the rudimentary rhizomycelium which acts as the absorbing system and upon which the sporangia and swellings typical of the genus are formed. Subsequently, there appears on the germ tube a fusiform swelling which normally becomes the incipient, primary zoosporangium. As this enlarges it occupies a considerable length of the germ tube whose branches become variously oriented upon it and function as rhizoids or branches of rhizomycelium. New branches may develop near the initial swelling and, as it grows, become included as part of the attached rhizoidal system. The empty zoospore case and the germ tube, which varies greatly in length, may soon collapse or be broken off from the developing thallus or may remain adherent well past maturity of the sporangium (fig. 25). After the establishment of the primary sporangial fundament, other swellings arise on the rhizomycelium, some of which are transformed into zoosporangia or resting spores, while many remain unaltered and are emptied of their contents as the thallus matures. The thalli are predominantly polycentric, but under certain as yet unknown conditions many monocentric thalli may be produced.

So many variations occur in the development of this chytrid that it is very difficult to describe any one type as definitely representing the normal. Consequently, a number of specific cases will be recounted from cultures on both cellophane and leaves grown under conditions described previously (Berdan, 1941a).

On cellophane, it is evident that a short, rather blunt germ tube is first developed (fig. 4, 11) and soon begins to branch. Apparently, this occurs whether or not the tube penetrates the substratum, although figure 9 illustrates a spore, lying in water above cellophane, from which three long, smooth and unbranched germ tubes have developed. Next appears the first swelling, which may be either the

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Contribution from the Botanical Laboratory of Columbia University, under the direction of Professor J. S. Karling.

incipient zoosporangium or a center about which growth is disposed. Figure 6 represents a germinating spore eighteen hours after inoculation of cellophane in a hanging drop. Around it lay many slightly swollen ($7\ \mu$) spores and a number with very short germ tubes. Most spores, however, bore tubes about $40\ \mu$ long and $0.2\ \mu$ wide, while a few measured $100\ \mu$ and had very long branches. Evidently, the rate of germination varies. The spore figured was kept under observation for several days. In figure 6 one incipient swelling is apparent on the germ tube. A day later (fig. 7) a second and larger swelling has arisen between this primary one and the first branches of the rhizomycelium, while further growth and new branches have been produced. The cytoplasm in the spore appears vacuolate at this stage and the globule of refringent material seems to be breaking up into smaller particles which are passing along the germ tube and into the swelling. On the third day, the primary swelling is seen to be greatly enlarged, and the wall is definitely double-contoured. The secondary and intercalary swelling, however, shows no further growth (fig. 8). Each swelling contains a single, large globule and the refringent material in the spore exhibits Brownian movement. On the fourth day, bacterial contamination obscured the observation of any further development.

Figure 13 pictures a young and more poorly developed thallus, whose one swelling, nevertheless, contains a large globule of refringent material while the spore is absolutely empty. There seems to be no correlation between the extent of the young rhizomycelium and the passage of refringent material from the spore.

In many instances, the rhizoidal tips penetrate the cellophane in from eighteen to thirty-six hours' time. Figure 20, however, illustrates a spore with a germ tube $160\ \mu$ long with a primary branch $100\ \mu$ in length and several secondary ones. There is a single swelling containing several refringent globules on the germ tube. Nevertheless, this rather highly developed thallus has secured no entry into the cellophane. Adjacent to it is the spore shown in figure 19, where the germ tube, which is but $32\ \mu$ long, has its tip firmly imbedded, and several swellings are developing upon it.

The extramatrixal zoospore may develop one to several extramatrixal germ tubes, and even a portion of the primary sporangium may be found outside the substratum (fig. 15a, 15b). In these figures the infecting germ tube is so short that the sporangial fundament seems to be adherent to the zoospore. Partially imbedded in the cellophane, it has caused this material to crack radially around it, and apparently some dissolution of the substratum has occurred. This is a very common appearance on cellophane and helps to locate the young thalli. In figures 5, 15a and 15b the initial swelling seems to have occurred along a branching portion of the germ tube so that a number of rhizoids have become laterally oriented upon it. A discussion of such rhizoid arrangement has been given in some detail by Karling

(1931a) for *Entophlyctis* and need not be described here. In figure 15a the swelling is still fairly fusoid and contains only a few globules, but in figure 15b, the sporangium is becoming irregularly shaped and the cytoplasm is filling with refringent material. Obviously, variation in shape is often determined at an early age. In both these figures many branches of the rhizomycelium could not be drawn, since they were imbedded in the cellophane above and below the sporangial fundament and the actual tips were obscured.

In figure 12a, most of the rhizoidal system between the spore and the branch bearing the sporangial rudiment, although swollen and constricted, is extramatrixal. In figures 12b and 14, the very long, narrow, smooth filaments are extramatrixal, while the intramatrixal ones are swollen, nodular, and constricted, which is very characteristic of *Septochytrium*. Many globules of refringent material are collecting in the primary swellings and travelling forward along the rhizomycelium. In figure 19 is represented a complete young thallus in which the cytoplasm is rather vacuolate, but glistening and shiny and containing a few refractive globules. The swollen and constricted character of the rhizomycelium is also clearly shown in this figure. Figure 21 illustrates a more advanced thallus, with a well-developed incipient sporangium. The rhizomycelium is imbedded in the cellophane and is fairly coarse ($2\ \mu$), while refringent material is accumulating in the sporangial rudiment.

In figure 10 is shown a portion of a young polycentric thallus after three days' growth in a hanging drop. The zoospore case is empty and beginning to collapse. Two rudimentary sporangia are forming and on the rhizomycelium, which measures $300\ \mu$ in length, are several fusiform swellings characteristic of this genus. Figures 22 and 23 show two thalli after three and seven days' growth, respectively. In the former, although it is still monocentric, the typical septations, constrictions, and swellings are becoming apparent. In the latter, the primary sporangium measures $26\ \mu$, the chief strand of rhizomycelium is $4.5\ \mu$ wide and several other branches are $4\ \mu$ in width. At least four more sporangia are in process of development and many constrictions, septations, etc., are indicated, although the full extent of the rhizomycelium is not shown because many fine branches are obscured by the cellophane in which they are imbedded and by their intermingling with branches of neighboring thalli.

Sometimes, during this period, a single sporangium enlarges and matures (fig. 25) so that a thallus of a simple monocentric rhizidiaceous type as in *Endochytrium* is produced. Other thalli become extensively polycentric, bearing branches with three and four sporangia in sequence. In these, the primary swelling frequently does not become a sporangium, but loses its contents, and to all appearances, remains functionless. Apparently, it has acted only as a temporary store-house or center from which protoplasm has migrated to new regions. These ini-

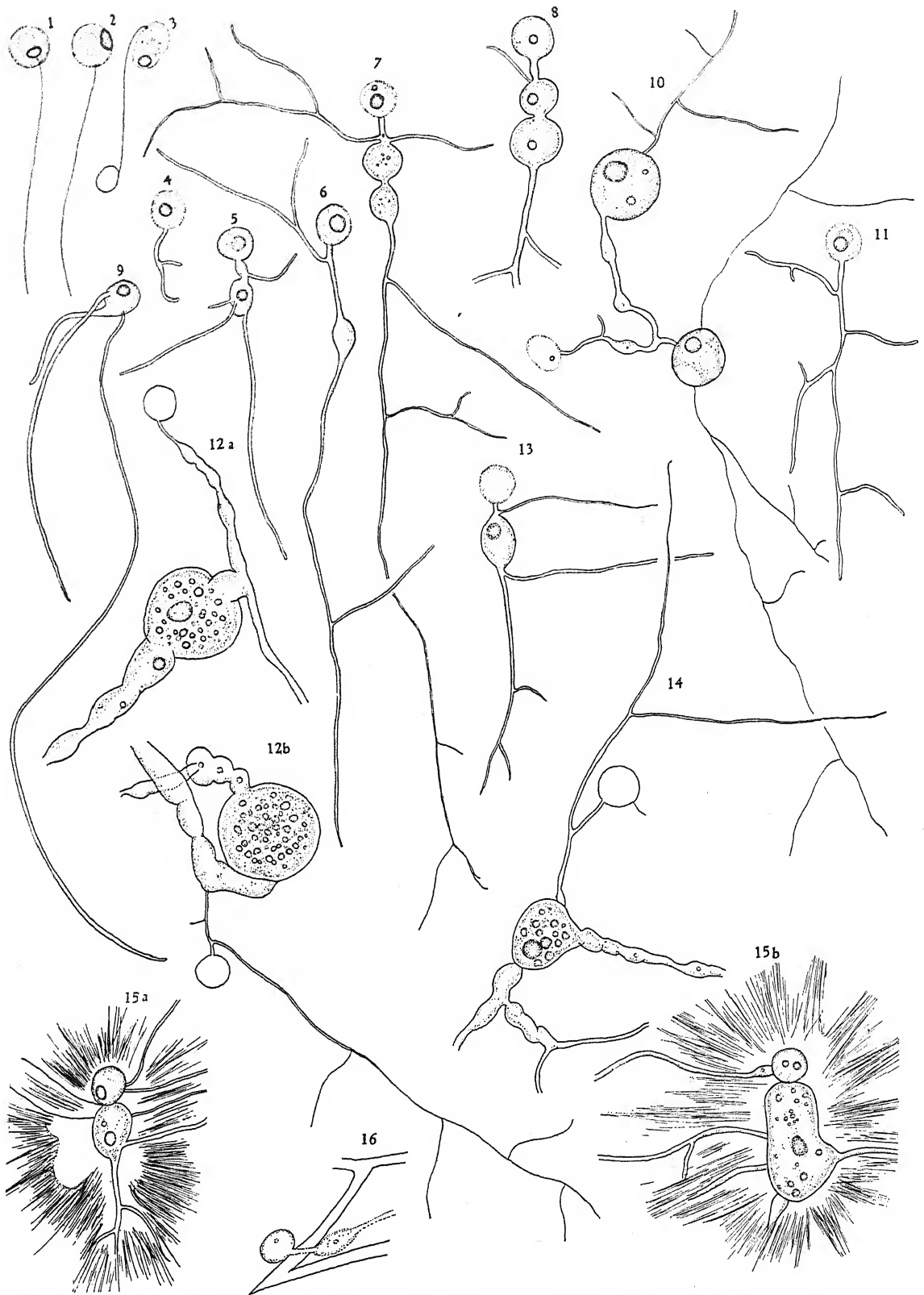


Fig. 1-16.—Fig. 1, 2. Zoospores.—Fig. 3. Zoospore showing loop on flagellum.—Fig. 4. Young germination stage in water above cellophane.—Fig. 5. Young stage of germination, showing primary sporangial fundament.—Fig. 6-8. Young

tial swellings may become very large and upon emptying turn brown and collapse.

In figure 26 is represented part of a mature polycentric thallus, in which the sporangia have been delimited from the rhizomycelium by the true cross walls and are both terminal and intercalary in position.

Infection of bits of sterile corn leaf in hanging drops gives similar germination stages, one of which is represented in figure 24. The germ tubes of zoospores frequently exhibit a form of tropism during their early development. Figure 17 illustrates a large, extramatrical sporangium with its rhizoids in cellophane, and which has shed its spores. Without exception, upon germination, the tubes have become oriented toward the substratum, and many have entered it. Two days later, only one tube was continuing to grow, even though the primary incipient swelling had been developed previously upon most of the others. This one (fig. 18) eventually developed a mature thallus with a sporangium.

When this chytrid was first discovered and grown in leaves of grass (July, 1938) and later in wheat leaves, the zoospores tended to accumulate near the stomata and the cut ends of the leaves. This is not uncommon and has been recorded for other chytrids (Cox, 1939; Haskins, 1940, etc.). From the spore, a fine germ tube then passed through the stomatal opening or penetrated the wall of the guard cell and developed the primary sporangial fundament in the leaf cells directly beneath. On the cut surfaces, the swellings developed near the point where the tubes entered the leaf. As a result, large groups of sporangia were found clustered about the stomata and along the ends of the leaf. The spore and the fine extramatrical portion of the germ tube usually shrivel up or are torn away so that these primary sporangia often appear to be terminal on the rhizomycelium. In tough leaves of certain varieties of corn, the spores become caught on the sharp spiky cells along the edge or on spiny epidermal hairs. Under such conditions, a great part of the rhizoidal system develops extramatrically, but the primary sporangial rudiment is always formed in the leaf cell (fig. 16). When such sporangia become very large, they fill the host cell and frequently distend it. The thalli might be either monocentric or polycentric under these circumstances.

Figure 27 shows a group of young thalli developing from spores near the stoma of a wheat leaf. It was drawn two days after the spores had been discharged into a new culture. Here, the nearly empty spore cases measure about $4.5\ \mu$, while the sporangial fundaments are about $8\ \mu$. The cytoplasm is hyaline

but glistening with a whitish gleam. A few strands of cytoplasm and a small portion of the refractive globule are still left in some spores. Vacuoles are not pronounced in the cytoplasm of the rhizoids which have an average length of about $100\ \mu$ and are branching.

A group of young thalli in the same leaf, drawn a day later (fig. 28) has greatly enlarged swellings and much heavier rhizoids. Several spore cases are still adherent. These swellings become sporangia, are cut off basally from the rhizoids by cross walls and at maturity develop broad necks which extrude through the stomata to open by an operculum and discharge their spores.

Although the thalli are commonly polyrhizoidal, they may often be monorhizoidal. The rhizoids or branches of rhizomycelium are oriented basally and laterally upon the sporangia as a rule (fig. 27-30), but when the sporangia are very large and centrally situated may often be so placed as to give a radiating appearance (Berdan, 1939, fig. 2). There are from one to twelve points of insertion, terminally placed sporangia often having but one, and intercalated sporangia but two, although here again a great variation exists. The extent of distribution and the size of the rhizomycelium (or rhizoids) is most unusual and comparable to that described for *Endochytrium operculatum* (Karling, 1937a) and *Rhizophlyctis Petersenii* (Sparrow, 1937; Haskins, 1940). The diameter of the rhizoids at the point of insertion on the sporangium ranges from 0.4 to $12.0\ \mu$, while the extent covers from $20\ \mu$ to $1\ \text{cm}$. Rhizoids extending into leaf tissue from sporangia formed near a cut edge are commonly very thick ($8-12\ \mu$) and remain so for long distances before beginning to taper. Those running parallel to and near by the midrib of a leaf tend to be straight and tubular and to follow in the long, narrow cells as a stout central axis with numerous branches. For example, one rhizoid near the mid-rib of a tough corn leaf extended $750\ \mu$ at a diameter of $8\ \mu$, giving off branches of varying width all along that length. Another filled the lumen of a fibre of lens paper for a length of $600\ \mu$.

At maturity, the rhizoids seem almost empty, containing but a few dark-colored globules and granules. The walls tend to become a light brown color. Earlier (fig. 29, 30), the hyaline cytoplasm is glistening, fairly homogeneous and contains small globules. The constrictions, partial septations, etc., that seem to be present in nearly all rhizoids during some stage of their development (fig. 22, 23, 26, 30, 41, 51) are so inconstant that it is almost impossible to give a representative description of them. True septa delimit the zoosporangia and resting spores from the rhizo-

developmental stages of the thallus during four days' time in a hanging drop.—Fig. 9. Spore germinating in water above cellophane to produce three long, smooth, unbranched germ tubes.—Fig. 10. Young polycentric thallus beginning to develop.—Fig. 11. Germinating spore showing branched germ tube 18 hours after inoculation of cellophane in a hanging drop culture.—Fig. 12a, 12b. Primary sporangium being developed. Note that the zoospore case and smooth narrow germ tube are extramatrical and adherent at some distance from the sporangial fundament.—Fig. 13. Young stage of germination with empty zoospore case. Refracting material is collected in a single globule in the sporangial fundament.—Fig. 14. Similar to 12a, 12b.—Fig. 15a, 15b. Young sporangia being developed in cellophane. The cellophane is cracking radially about the thallus.—Fig. 16. A zoospore germinating on a spiny cell at the margin of a wheat leaf.



Fig. 17-26.—Fig. 17. Spores from the empty sporangium beginning to germinate. The germ tubes exhibit tropic movement in the direction of the substratum, cellophane.—Fig. 18. The single spore from figure 17 which continued to develop. Eventually a thallus with a sporangium matured from this.—Fig. 19. Young thallus in cellophane.—Fig. 20.

mycelium at maturity and often appear convex (fig. 41, 51). Whether or not septa are present in other positions along the rhizomycelium and rhizoids is still somewhat questionable, but bands or trabeculae of some opaque material extend partially or entirely across the width of the filaments in many places (fig. 31, 32, 41, 50, 51). They are not infrequent at the point of branching and may be transformed remnants of cytoplasm and refringent material rather than actual septa. In some rhizoids or even in whole thalli, very few constrictions or swellings occur, while in others they are numerous and arranged either in series or at intervals of about $10\ \mu$. Even in a single spore strain there is such a range of variation that it becomes difficult to distinguish that which is typical.

The swellings of the rhizomycelium are comparable to the spindle organs, "Sammelzellen," etc., of other genera of the Cladochytriaceae, except that there is a much wider range in size and shape. They are non-septate, continuous with the rhizomycelium and formed singly or in conjoining series. In the latter case, they seem to be separated by opaque bands similar to those elsewhere throughout the rhizomycelium, and rhizoids may often originate upon them. The swellings are commonly spherical, ovoid or fusoid in shape, but polyhedral, turbinate or irregularly shaped ones are not uncommon. In size, they are $2\text{--}100\ \mu \times 2\text{--}200\ \mu$ (usually $15\ \mu \times 20\ \mu$). The extremely large turbinate to polyhedral or irregular organs result chiefly from primary swellings which lose their contents before zoospores can be formed. In one such instance a large ($50\ \mu \times 100\ \mu$), thin-walled organ gave off twelve branches of rhizomycelium. These extended over $1,500\ \mu$ without taking into account the finer rhizoidal branches. Large ($40\text{--}75\ \mu \times 50\text{--}125\ \mu$) primary sporangia, which were spherical, ovoid or pyriform in shape, formed on five of them. Other secondary and tertiary sporangia as well as many ordinary swellings were also produced. One branch of the rhizomycelium bore four sporangia while another produced none at all. In a second case, two branches of rhizomycelium given off from such a swelling produced eleven sporangia. It seems to the writer that extremes of polycentricity may result when the original swelling does not become a zoosporangium, but the contents migrate from it after it has reached large proportions. At first, the swellings contain clear, shiny cytoplasm and refringent globules. Later, they either are empty or retain small amounts of dark material in more or less globular condition. Occasionally, they seem to have divided into two cells, one of which forms a zoosporangium or resting spore while the other remains attached as a sort of apophysis.

The development of the zoosporangium is essentially like that recounted many times previously for various chytrids and particularly as outlined for *Endochytrium operculatum* by Hillegas (1940) so it need not be described again. The alveolar stage described for *Catenochytridium* (Berdan, 1941b) is also found in many of these sporangia and seems to persist for a longer time period. This condition has been described and figured for *Nepbrochytrium stellatum* (Couch, 1938) as a normal part of the internal changes during sporangium development. Here Couch illustrates the continued presence of such vacuoles during all the stages of coalescence of the refringent material. This same condition has been noted frequently in *Septochytrium*, especially in long, narrow sporangia, but positive evidence has not been secured that normal spores are later discharged from these sporangia. One sporangium under observation required $10\frac{1}{4}$ hours to pass through all the internal changes from the time it was delimited from the rhizoids until all the spores were discharged.

The shapes and sizes of sporangia have been given previously by the writer (1939) and are illustrated here in figure 51. Small sporangia are found which contain but one zoospore. Others are so large that they can be observed in the substratum with the naked eye. In flask-shaped, pyriform, tubular, etc., sporangia some distinction seems necessary between the sporangium proper and the "exit papilla" or "neck" at the tip of which the operculum is formed. Since, at maturity, the whole interior of the sporangium, regardless of its shape, becomes filled with spores, it seems reasonable to consider only the hyaline region directly beneath the operculum as the exit papilla. When lobes and projections of sporangia are not special structures for the exit of zoospores and are not set apart in any way from the remainder of the sporangium, they should not be termed "exit tubes." Nevertheless, every sporangium bears a short, broad, papillate portion which extends to the exterior before the operculum opens. In leaves, it may crowd out through a stoma. In cellophane, the imbedded part often has a brown wall while the extramatrix portion remains much paler in color. When the rudiment forms just below the surface of the substratum which is afterward ruptured by the large and swelling sporangium, the latter may seem to become completely extramatrix in situation, and many rhizoids extend out into the surrounding water.

The region in which the operculum will form is marked out as a hyaline area just below the wall of the exit papilla. Under high magnification, the wall of the sporangium seems to be made up of more than

Extension germ tubes, one branch bearing a swelling, lying in water above cellophane.—Fig. 21. Similar to 19, but more advanced.—Fig. 22. Similar to 21. The thallus is becoming extended and typical swellings and constrictions are developing on the rhizomycelium.—Fig. 23. A stage of development later than that in figure 22. The thallus is polycentric and rhizomycelium coarse, swollen and constricted with trabeculae or false septa extending across its width.—Fig. 24. Young stage of thallus from a zoospore germinated on a corn leaf in a hanging drop.—Fig. 25. A monocentric thallus. The sporangium is maturing but the zoospore case is still adherent.—Fig. 26. Polycentric thallus producing five sporangia. Two have shed their spores.

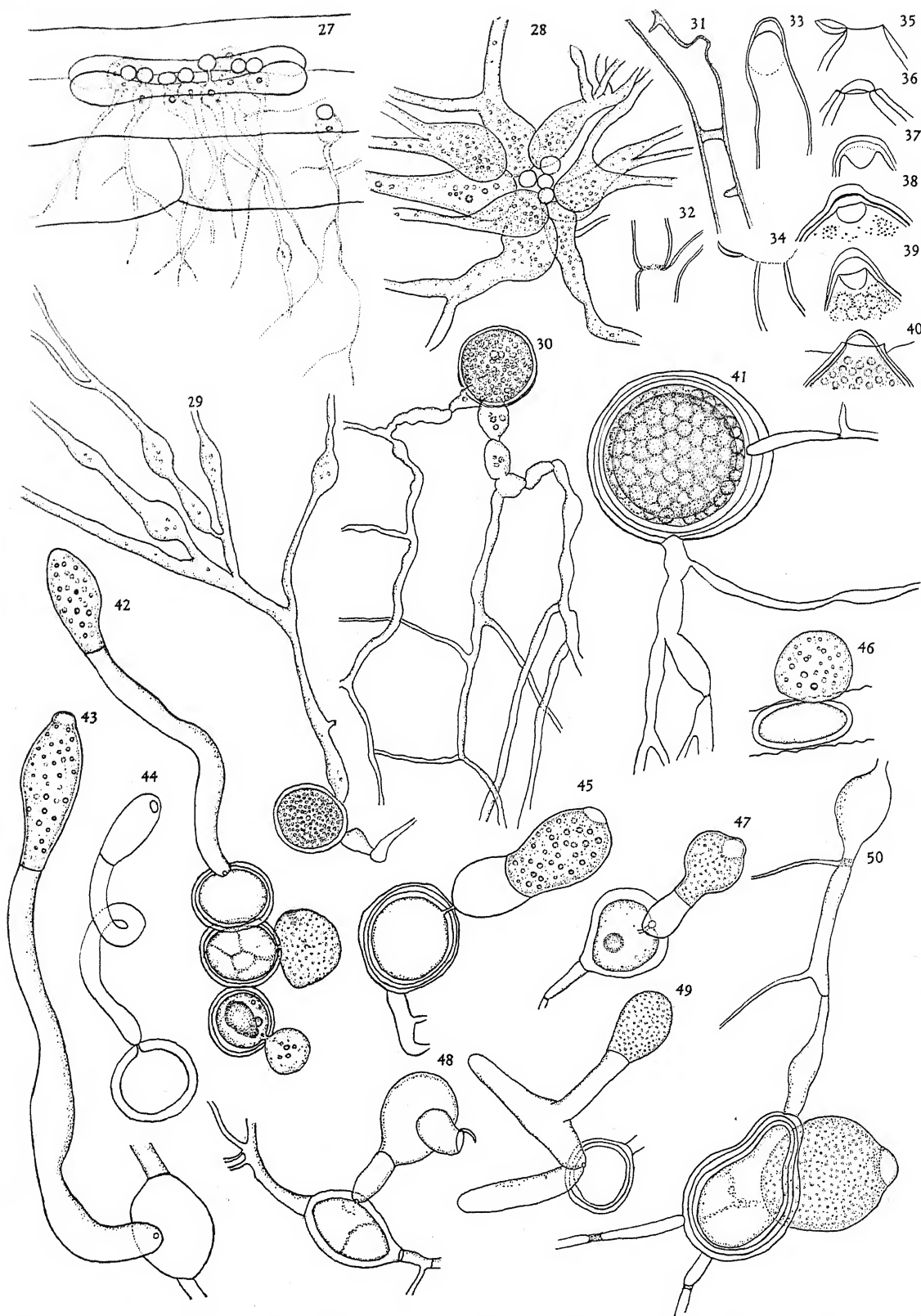


Fig. 27-50.—Fig. 27. Group of young thalli from spores whose germ tubes have entered through a stoma of wheat leaf.—Fig. 28. A stage two days later and from the same leaf as in figure 27. Note increase in size of sporangial funda-

one layer. The outer one apparently thickens to form the "cap" (operculum) while the inner one seems to swell or bulge inwardly (fig. 35, 37, 38, 39). Karling (1937a) has described this region in *Endochytrium operculatum* as being filled with a viscid hyaline fluid and such may be the case here, although figures 33, 37, 38, 39 seem to indicate some connection with the wall. Plasmolysis of this region does not cause this area to shrink back with the plasma membrane. Figures 36 and 40 illustrate two puzzling cases where the outer layer of the wall appears to have broken off or to be abruptly truncated, while the inner layer and sporangial contents protrude through the opening.

In figure 34 is shown the sequel to figure 33. The operculum has been pushed off but is still attached to the sporangium by a very fine membrane. The first sign that the operculum is about to be forced off is the appearance of a thin ring in the outer layer of the wall just below the thickened "cap." Sometimes the lid is actually separated from the rest of the sporangium by this thin area as is indicated by two fine lines ringing the "neck" of the sporangium. It may remain hinged to the sporangium but usually is carried upward by the emerging spores. Although many sporangia with branches or extended arms have been seen (fig. 51), especially in old tough corn leaves and cellophane, only once has a sporangium produced two opercula. The orifice is circular, $1-16\ \mu$, or oval, $4-6\ \mu \times 6-10\ \mu$. The first material to emerge after dehiscence is clear and possibly represents the hyaline content present in the region just beneath the operculum. When the operculum is attached and lying below the exuding spores, where it acts like a rounded gang-plank, the spores slide down over it two or three abreast. Their long flagella trail behind as fine dark lines and make channels into which the succeeding spores must crowd as they escape. The effect is of striations on the sporangial wall until careful observation interprets it otherwise. The sporangial wall does, indeed, become brown and wrinkled with age but not until later. Sometimes, the crowding spores are squeezed into hexagonal patterns. Again, some may be very amoeboid or stretched out into long, narrow shapes as they reluctantly make an exit. This happens frequently when sporangia are borne among the meshes of fibers in lens paper. Then the spores become entangled and exhibit striking amoeboid and elastic movements. From small sporangia the spores are usually extruded all at once in a globular mass which remains quiescent for a few moments. Then the mass loosens up and spores begin to dart out of it. When large numbers (75,000) of spores are discharged from a single sporangium, the first ones form a ball, and later ones are hampered in their exit by its presence and must either push it aside or stream around it to form irregular masses nearby which may cover areas

of $100\ \mu$. Nevertheless, these, too, remain quiet before swimming away. Laggards have great difficulty in escaping, since the opening is choked by the preceding spores, so that germination "in situ" is common. Many of these trapped zoospores go into a resting condition and upward of 75 small resting spores may be counted in an old sporangium. Then it is not possible to ascertain the method of germination of the zoospore. Hillegas (1940) reports a similar phenomenon for *Endochytrium operculatum* and fails to find evidence of rhizoids but considers that the zoospores have enlarged directly. The time required for exit of the zoospores naturally depends upon the size of the sporangium. The spores are usually swimming actively within three minutes of their discharge.

RESTING SPORE DEVELOPMENT AND GERMINATION.—Resting spores are commonly developed when conditions are unfavorable for zoospore production or discharge. They may form from any of the swellings on the rhizomycelium from which zoosporangia are normally produced and in any of the positions described previously for the latter structures.

The internal changes which take place during the formation of the resting spore of *Septochytrium* are essentially similar to those described and figured many times previously for various chytrids and are, therefore, not recounted again in detail nor illustrated. The first sign that a resting spore is about to develop is the increase of the refractive material in a swollen sporangial rudiment, unaccompanied by further enlargement of the cell or signs of zoospore formation. As the increase continues, the refringent material fuses into large, irregular globules. Then the wall begins to thicken and to turn brown. Finally, one large globule of brownish, oily substance is formed, while around it in the peripheral cytoplasm, a layer of small globules (fig. 41) which may be packed closely into a surface, hexagonal pattern, is produced. At maturity the wall is layered (fig. 41, 45, 50), $1.5-4.0\ \mu$ thick, and pale amber to dark brown in color. The outer layer is not always of equal thickness throughout and although generally smooth, may be somewhat roughened or uneven. Adjacent branches of rhizomycelium often tend to develop brown and thickened walls, also.

The resting spores are spherical, ovoid, pyriform, flask-shaped or much elongated in shape and $4-60\ \mu \times 4-65\ \mu$ in size (fig. 52). Up to the present no evidence of sexuality has been seen in connection with the production of resting spores.

At germination, the resting spore functions as a prosporangium. The preliminary changes in the content prior to germination are primarily the same as those described many times previously for various chytrids (Berdan, 1941a). However, the resultant zoosporangia may be in direct contact with the resting spore through the pore in its wall (fig. 42, 46), or they may be formed at the end of a tube produced

ments and width of rhizoids.—Fig. 29, 30. Portions of young thalli. The primary sporangial fundament has become thick-walled while constrictions and swellings are forming in the rhizomycelium.—Fig. 31, 32. Portions of the rhizomycelium showing opaque bands which pass entirely or partly across its width.—Fig. 33-40. Views of operculum in various stages.—Fig. 41. A mature resting spore.—Fig. 42-50. Stages and variations in resting spore germination.

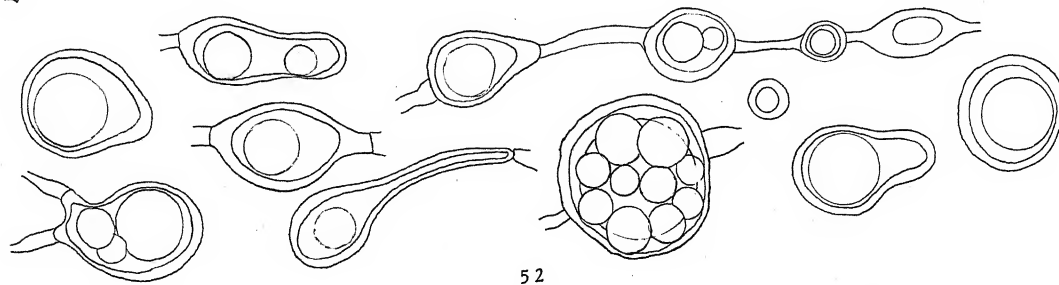
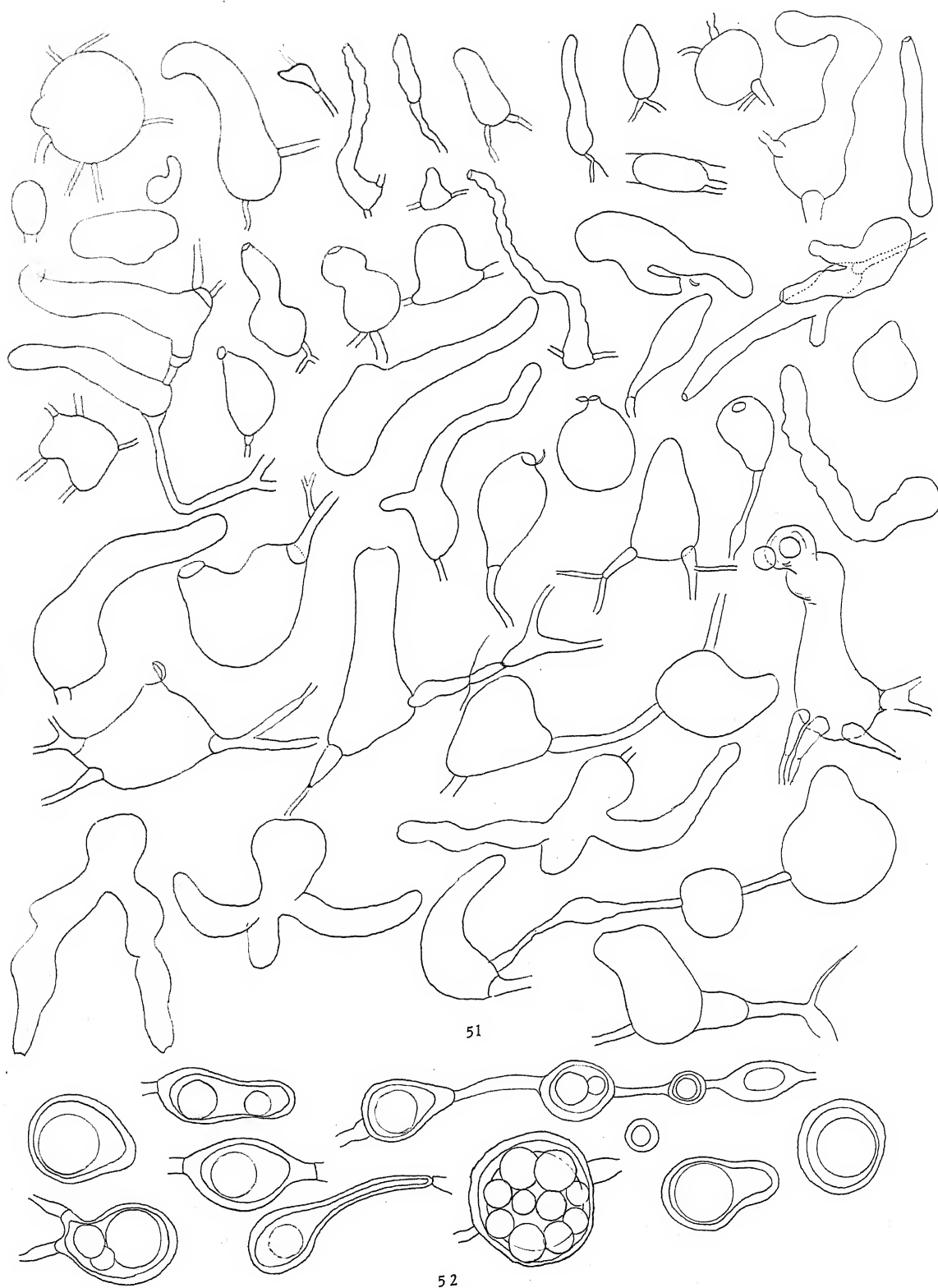


Fig. 51-52.—Fig. 51. Outline drawings to indicate range in size and variation of the zoosporangia.—Fig. 52. Outline drawings illustrating various shapes of resting spores. Note position of the large globule.

by the germinating spore (fig. 42-45 and 47-50). As the tube gradually elongates, the protoplast of the spore empties into it and eventually concentrates in the tip to form an operculate zoosporangium which is delimited by a cross wall. The first type of zoosporangium mentioned above is usually spherical, pyriform or ovate while the second variety is oval, rounded pyriform, clavate, obclavate, etc. In size they are proportionate to the resting spore from which they are derived. The tube may be wide and sac-like (fig. 45) to very long and narrow (fig. 43) when it may have twists, coils (fig. 44) or branches (fig. 49). According to a preliminary paper, it is $7-26 \mu \times 10-450 \mu$ in size. Since then, using cellophane as a substratum, tubes as narrow as 2μ and as long as 700μ have been found.

The resting spores were first seen to germinate in the laboratory at Columbia University after an air-mail trip in stoppered test-tubes from London, Canada. Since then, germination has occurred a number of times but never again in such quantity.

DISCUSSION.—This genus has been placed in the family Cladochytriaceae, since it is predominantly polycentric and possesses a rhizomycelium bearing numerous rhizoids and terminal and intercalary swellings which may at maturity be transformed into zoosporangia or resting spores. As it stands at present, there is but one valid operculate genus, *Nowakowskiella*, in this family. According to the description as given by Schroeter (1897), the zoosporangia are usually apophysate and proliferating one to several times. While sporangia of *Septochytrium* occasionally arise in such a manner as to appear apophysate, no proliferation has been seen up to date. There are but two established species of *Nowakowskiella*, and one other whose validity is questionable. *N. elegans*, the type species, has been grown by the writer a number of times and has a much more delicate and tenuous rhizomycelium than *Septochytrium*. The measurements of zoosporangia as given by Nowakowski (1876), Schroeter (1897), Matthews (1928), and Sparrow (1933) are also much smaller than in the present species, while the presence of resting spores has not been ascertained with certainty. *N. ramosa* (Butler, 1907) has a unique type of resting spore which has been discussed thoroughly in connection with *Cladochytrium hyalinum* (Berdan, 1941a) and resembles that of *Septochytrium* in no way. Since *N. endogena* has been figured by Constantineanu (1901) as monocentric and lacking intercalary swellings, it cannot justifiably be included in the genus as it is described at present. Nevertheless, there is a slight possibility that our fungus is a new and hitherto undescribed species of *Nowakowskiella*, although it disagrees with the current diagnosis of that genus.

A new genus, *Megachytrium*, was established by Sparrow (1933) for a chytrid which has occasionally apophysate sporangia, a thick and sometimes septate mycelium and terminal or intercalary swellings. Yet it lacks rhizoids and the swellings are cut off from the rhizomycelium by cross walls, which

seems to separate it effectively from *Septochytrium*. *Endochytrium ramosum* as described and figured by Sparrow (1933) is a polycentric form with a profusely branched rhizomycelium bearing rhizoids and operculate zoosporangia but lacking swellings. No formation of resting spores has been observed for this species. The monocentric species, *E. operculatum*, resembles monocentric thalli of *Septochytrium* in the variation of shape, size, etc., of sporangia, resting spores, and type of development of the thallus. Hillegas (1940) has reported an instance where two resting spores were developed on the same thallus of *E. operculatum* and suggests that this may indicate a trend toward polycentricity. *Septochytrium* is often monocentric. Perhaps these combined data may suggest some evolutionary connection between the genus *Endochytrium* of the Rhizidiaceae and the operculate genera of the *Cladochytriaceae*. Certainly the limits of the two families do not seem to be quite so sharply defined as previous diagnosis might lead us to believe, and in some genera the cause of duplication of polycentricity of the thallus may be more environmental than inherent.

In consideration of the findings of Karling (1937b) and Hillegas (1940) for *Cladochytrium replicatum* and *Endochytrium operculatum*, respectively, a cytological study of nuclear behavior in *Septochytrium variable*, where the percentage of monocentric thalli seems fairly high, might be of considerable interest and illumination as regards transition from the rhizidiaceous form of thallus to the polycentric type found in the *Cladochytriaceae*.

SUMMARY

The details of the life history, with particular emphasis on germination stages in cellophane, are described for this chytrid, recently reported and diagnosed as a new genus.

It is extremely variable, so the isolation of a strain from a single spore was deemed necessary and the description is taken from this strain.

The genus is placed in the family Cladochytriaceae because of its predominantly polycentric rhizomycelium with numerous swellings from which the zoosporangia and resting spores are developed.

The sporangia are operculate but rarely apophysate. Consequently the relation to *Nowakowskiella* and *Megachytrium* is discussed.

The range in variation of size and shape of sporangia and resting spores and in development of the thallus is very wide.

No evidence of sexuality is found in the development of the resting spore.

At germination, the resting spore functions as a prosperangium. Zoosporangia may be formed in direct connection with the spore by means of a pore in the wall, or at the end of a tube produced from the resting spore. The tube may be saccate and broad, or extremely long and narrow, straight, coiled, twisted or branched.

Polycentric thalli are unusually extensive.

Monocentric thalli are often formed. In size and general characters these are similar to *Endochytrium operculatum* Karling but the rhizoids do not conform accurately to the description in that genus.

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DISTRIBUTION OF STRUCTURAL HYBRIDS IN *PAEONIA CALIFORNICA*¹

James L. Walters

THE OCCURRENCE of structural hybridity in the chromosomes of *Paeonia* subg. *Onaepia*, owing to extensive reciprocal translocation and inversion, was first reported by Stebbins and Ellerton (1939). This subgenus is composed of the two American species, *P. californica* and *P. Brownii*, the former inhabiting coastal southern California and the latter a portion of montane western North America (Stebbins, 1938a). Stebbins and Ellerton thoroughly analyzed the ring configurations found in collections of *P. Brownii* from two localities and of *P. californica* from one. The present paper will deal with the broad aspects of the distribution of structural hybrid features of *P. californica* throughout its range. A more complete analysis of structural hybridity in the chromosomes of both species will be furnished in a later publication.

MATERIAL AND METHODS.—Material of *P. californica* was collected in various parts of its range, extending from the northernmost known station (near King City, Monterey County) to points along the border between San Diego County and Lower Cali-

fornia. The latter is not far from the southernmost known locality. The collections in most regions also include the farthest inland extensions of the range. The accompanying map (fig. 10) shows the distribution of the collections throughout the range of the species. Flower buds were fixed in 3:1 absolute alcohol-glacial acetic acid overnight, then washed and stored in 70 per cent alcohol. Anthers were softened by placing in iron aceto-carmin for fifteen minutes in the paraffin oven (54°C.) and then smeared in the usual manner. The studies of meiotic configurations were made using a 90× apochromatic objective with 15× compensating oculars. The photomicrographs (fig. 1-6) were taken with the same optical equipment, and a Leitz Makam camera.

TYPES OF STRUCTURAL HYBRIDS.—The chromosome number in *P. californica*, as in most other species of the genus, is $n=5$. All five chromosome pairs have been identified in somatic tissue and given letter designations (Stebbins, 1938b). In meiosis, the three pairs with median centromeres cannot be distinguished from one another, and are labelled *M*, but the submedian (*D*) and the subterminal (*E*) pair are readily recognized in most cells (fig. 1-4). It should be emphasized that the recognition of these chromo-

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some types does not necessarily imply strict homology between similarly lettered chromosomes in the various species, or even between "homologous" chromosomes in the same plant of *P. californica*, as pointed out by Stebbins and Ellerton (1939). Since extensive reciprocal translocation has taken place in this species (and the related *P. Brownii*), it is clear that homology exists only in terms of parts of chromosomes. In certain plants it has been possible to show that unequal translocation has altered the relative lengths of chromosome arms so that in these plants the type of a given chromosome may be uncertain (Stebbins and Ellerton, 1939, fig. 2). Such changes in homology have also been studied by Sax (1931) in *Rhoeo discolor*, and he has pointed out that it is not possible to match all of the twelve chromosomes of this species into homologous pairs.

The chromosome configurations now known in *P. californica* include all combinations of pairs and rings possible with ten chromosomes. These configurations are as follows: 5_{II} (fig. 1); $\odot 4^2$; $\odot 6$; $2 \odot 4$ (fig. 2); $\odot 8$ (fig. 3); $\odot 6 + \odot 4$; and $\odot 10$ (fig. 4). The studies of Belling and Blakeslee (1926) and many others have shown that the existence of such rings of chromosomes in meiosis is due to reciprocal translocation between non-homologous chromosomes. This gives rise to chromosomes each of which is homologous with two other chromosomes, one at each end. The process is too well known to require further elaboration here. One reciprocal translocation produces a $\odot 4$. A second translocation produces a second $\odot 4$ if it involves none of the four chromosomes in the first ring; or a $\odot 6$ if any of these four is involved. A third translocation in either a $\odot 6$ or a $2 \odot 4$ plant results in a $\odot 8$ or a $\odot 6 + \odot 4$ type, depending again on the chromosomes involved in the new translocation. A fourth translocation produces the complete $\odot 10$. It should be noted that these are the *minimum* numbers of translocations required to produce each of the rings just described; other translocations (termed "redundant" by Darlington and Gairdner, 1937) may occur, which do not increase the size of the ring.

There are of course more than seven types of configurations with respect to the arrangements of their chromosome segments, owing to the fact that a ring containing a given number of chromosomes may in different plants include different chromosomes. To some extent, these differences can be detected, since two of the pairs may be distinguished from the other three and from each other. Thus, a plant with $\odot 4$ collected at Pozo, San Luis Obispo County, has four *M* chromosomes in the ring; a $\odot 4$ type from west of Pasadena, Los Angeles County, has two *M* chromosomes and the two *E* chromosomes in the ring; one from Sepulveda Canyon, Los Angeles County, has two *M* chromosomes and the two *D* chromosomes in the ring. This represents all the differences that can be detected cytologically in the ring of four. Two of these are shown in the $2 \odot 4$ type from Altadena (fig. 2). Similar differences have been recognized

² The symbol \odot means "ring of."

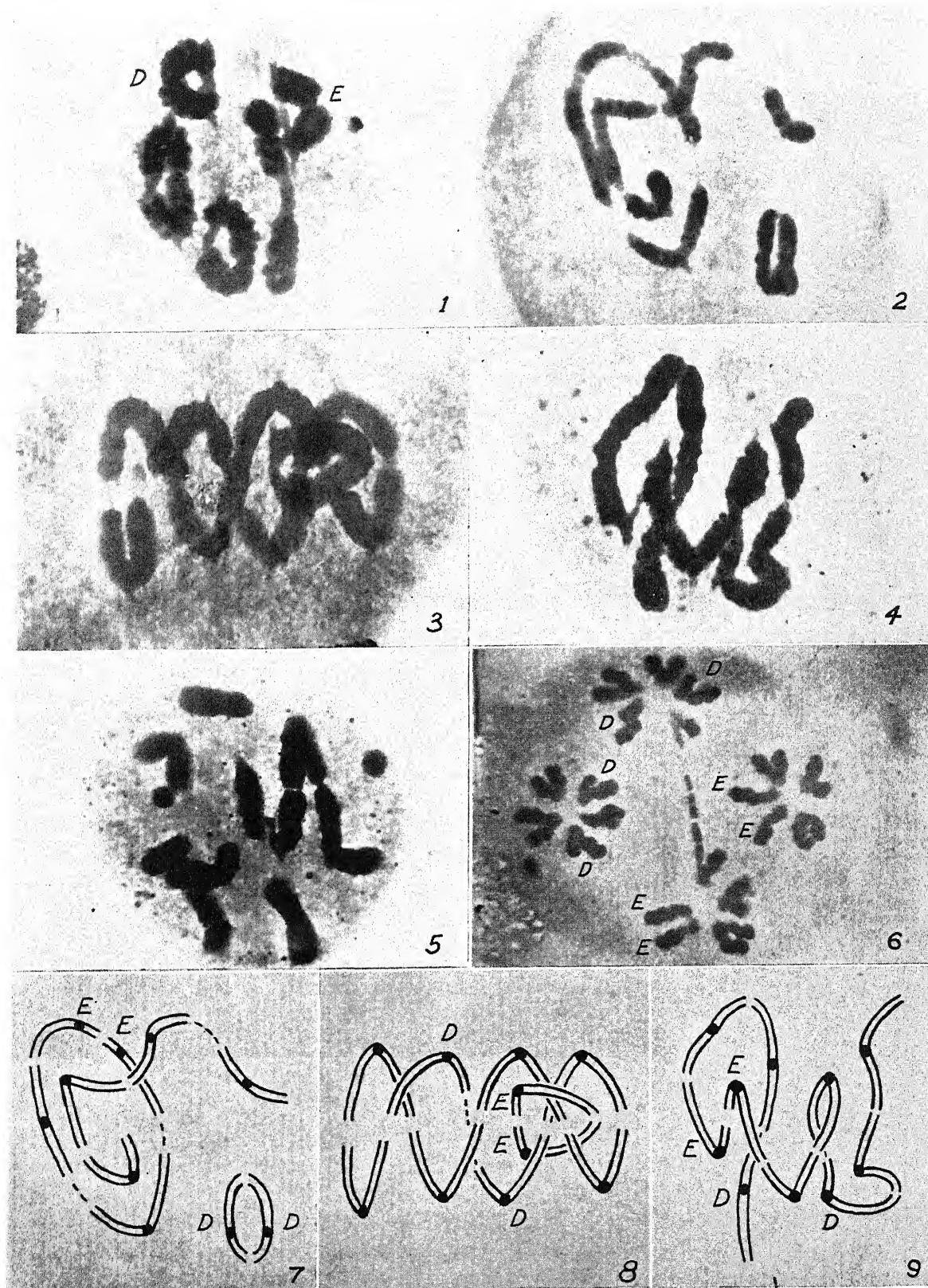
in the larger rings, and the complete data will be presented in a later paper.

This complete series of structural hybrids parallels the situation known in *Oenothera* subg. *Onagra* (Cleland, 1936, and others), and exceeds in extent any of the other reported cases of naturally occurring structural hybrids (Darlington and Gairdner, 1937, on *Campanula persicifolia*; Sax, 1931, on *Rhoeo discolor*; Hoar, 1931, on *Hypericum punctatum*; Kattermann, 1938, on *Briza media*).

INVERSIONS.—The occurrence of extensive inversion hybridity was mentioned by Stebbins and Ellerton (1939), and only a brief confirmative mention will be made here. Inversions are so extensive as to be ubiquitous; the author has never observed a plant which did not show at least a small percentage of the familiar bridge-fragment configurations in first or second anaphase. These will be reported on more fully in a later publication. A rather striking configuration is shown in figure 6, where a "diagonal" bridge may be seen at second anaphase. In this cell, non-disjunction has taken place at first anaphase, as two *E* chromosomes may be seen in two of the anaphase groups and two *D* chromosomes in the other two. The way in which the bridge is related to the non-disjunction shows clearly that the bridge is a surviving first-anaphase bridge, and not the result of disjunction of a loop chromatid (Darlington, 1937, pp. 265-9). It is also clear that the inversion is in an *M* chromosome. The fragment may be seen adjacent to one of the chromosome groups.

DISTRIBUTION OF THE RING TYPES.—It has been mentioned that the collections reported on here cover a representative part of the entire range of the species, including the northern, southern, and inland limits. The geographic distribution of the structural hybrid types throughout this region shows an interesting feature: there is a tendency for plants occurring near the limits of the range of the species to be more highly heterozygous than those growing near its center of distribution. This tendency is shown on the map (fig. 10), where the different types are shown by symbols. In San Diego County, only the higher heterozygotes are found: $\odot 8$; $\odot 6 + \odot 4$; and $\odot 10$. These types are also known from Riverside County, with the addition of $2 \odot 4$. At the northern extreme, in Monterey County, high heterozygosity is also found, though it is less striking here than at the southern extreme just described. In Monterey County $\odot 8$ is known from two collections, and $\odot 6$ from one. In the intermediate areas, though high heterozygotes are present (the highest being $\odot 8$), the proportion of homozygotes and low heterozygotes is large. This is particularly clear in San Luis Obispo and Santa Barbara Counties; homozygotes will also be noted in Ventura, Los Angeles and Orange Counties, where, however, fewer collections have as yet been made.

It is not to be implied from the above description that any one area is always homogeneous as to the ring types it includes. *Paeonia californica* grows, in most areas studied, in clearly defined colonies of



usually not more than a hundred yards in extent, with intervals between colonies ranging from a half mile to many miles. The majority of the collections were made by taking at random ten plants of each colony. In some cases, examination of the ten plants has shown remarkable heterogeneity within the colony. For example, the collection from west of Pasadena, Los Angeles County, shows the following distribution: 1 plant with 5_{II} ; 4 plants with $\odot 4$ (MMEE); 2 plants with $\odot 4$ (MMMM); 2 plants with 2 $\odot 4$ (MMMM + MMEE); and 1 plant with $\odot 6$ (MMMMDD). The collection (8 plants only) from San Juan Hot Springs, Orange County, is interesting in that no single-translocation form ($\odot 4$) was found: included are 4 plants with 5_{II} ; 3 with $\odot 6$; and 1 with 2 $\odot 4$. On the other hand, a colony collected just north of Altadena (about 5 miles from the colony west of Pasadena) is found to be entirely homogeneous, showing 2 $\odot 4$ in all ten plants collected. The heterogeneity of the various colonies will be described more fully in a later publication. The information obtained up to the present time indicates that the maximum variability in any one colony does not exceed two translocations (the difference between 5_{II} and $\odot 6$, for example).

DISCUSSION.—A comparison may be made between the regularities just noted in the distribution of *Paeonia californica* structural hybrids and certain features of the distribution of American races of *Oenothera* subg. *Onagra* (Cleland, 1940). In the latter group, the regularities are perhaps more striking. California is occupied by the *hookeri* alliance, composed chiefly of forms with 7_{II} or small rings. In New Mexico the *irrigua* alliance is found, characterized by rings of intermediate size such as $\odot 6$, or $\odot 6 + \odot 4$, or $\odot 8$. The *Jamesii* of Oklahoma show a similar situation. In the eastern part of the range, practically all races show the complete $\odot 14$. The races of the *Rydbergii-strigosa* assemblage of the northern and eastern Rocky Mountains and the great plains also show complete rings. This completely heterozygous type, then, occupies the major portion of the range of *Onagra* in North America.

This predominance of the complete ring in *Onagra* suggests that this type has a higher survival value than the smaller rings or the structural homozygotes, and Cleland (1940), following Darlington (1929), has advanced a likely explanation of this advantage. Briefly, he views the complete ring as a mechanism

for ensuring maximum heterozygosity (and thus heterosis) under self-pollination, since a single pair of balanced lethals is in this case sufficient to preserve heterozygosity. The pair of lethals reduces fertility by 50 per cent, but "with heterozygosity of all or practically all the chromosomes thus preserved, cross-pollination would no longer be of value, and the increased certainty of seed setting resulting from self-pollination would probably more than make up

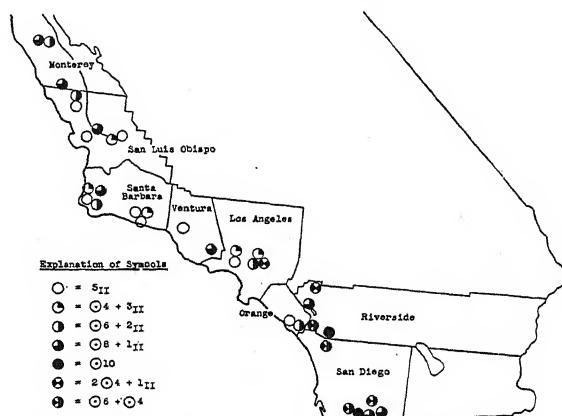


Fig. 10. Map of the southern half of California, showing the counties in which *Paeonia californica* has been collected, and the distribution of the structural hybrid types. Further explanation in the text.

for the sterility due to the lethals" (Cleland, 1940). In *Paeonia californica*, the distribution of the various ring types does not show the clear-cut differences reported by Cleland for *Onagra*, nor does the completely heterozygous type occupy the major portion of its range (as the map shows). Further, no area within the range of *P. californica* is exclusively occupied by the complete ring, in sharp contradistinction to *Onagra*. In the related *P. Brownii*, however, there is some indication that the *Onagra* situation is more closely approached; more complete data must be obtained before any definite statement is possible.

From present evidence, therefore, it does not appear that any one type of configuration in *P. californica* is significantly more abundant than any of the others, and thus it is not possible to say that in this species the complete ring, or any other configuration, has a superior survival value. There are two

Fig. 1-6. Photomicrographs of meiosis in *Paeonia californica*.—Fig. 7-9. Diagrams of figures 2-4, respectively.—Fig. 1-5 and 7-9, $\times 1,300$; fig. 6, $\times 950$. All from acetocarmine smears.—Fig. 1. Structural homozygote (5_{II}); late metaphase. The submedian (*D*) chromosome pair and the subterminal (*E*) pair are clearly distinguished from the other three (*M*) pairs. San Juan Hot Springs, Orange County.—Fig. 2. 2 $\odot 4 + 1_{II}$; prometaphase. Failure of chiasma formation causes one ring to appear as an open chain. Altadena, Los Angeles County. Diagrammed in figure 7.—Fig. 3. $\odot 8 + 1_{II}$; late metaphase. The pair is interlocked with the ring; the chromosomes of the ring are alternately distributed. The significance of the interlocking is discussed in the text. Los Alamos, Santa Barbara County. Diagrammed in figure 8.—Fig. 4. $\odot 10$; early metaphase. Failure of chiasma formation in one arm causes the ring to appear as a chain. Temecula Grade, Riverside County. Diagrammed in figure 9.—Fig. 5. Two fragments at metaphase in the same plant as figure 4. The smaller fragment is attached to an *M* chromosome by a terminal chiasma, and just below it is a short chromosome from which one or both fragments are derived. Further discussion in the text. The chiasma frequency in this cell is low; in this respect it is more typical than figure 4.—Fig. 6. Bridge-fragment configuration at second anaphase in the same plant as figures 4-5, the result of an inversion. Non-disjunction has taken place at first anaphase in the *D* and *E* chromosomes. Further explanation in the text.

suggestions that may be made at the present time regarding the causes of the distribution of the different types, which must await further evidence for confirmation or disproof.

One is that the present "center of distribution" of the species is also its center of origin, since the structurally homozygous types are more abundant there and absent from the peripheral portions of the range. This suggestion, however, is not supported by the geologic history of the region (Reed, 1933). The oldest land area in the range of the species is in and around San Diego County, where the largest rings are found. This region has been land continuously since the Cretaceous, and there is good reason to believe that *Paeconia*, an ancient group, has inhabited the region uninterruptedly at least since the Oligocene or Miocene epoch. San Luis Obispo and Santa Barbara Counties, now apparently the center of distribution, were inundated during the upper Miocene, along with Monterey County, and in part during the Pliocene. Therefore, it is possible that the species radiated northward from its present center in Santa Barbara County to its present northern limit; but it is very unlikely that the southern portions were colonized in this manner, as the southern portions were probably occupied by *Paeconia* earlier than this present center.

The other suggestion is that some of the higher heterozygotes have colonized those portions of the range which are ecologically more extreme. Thus, the rainfall in San Diego and Riverside Counties is the lowest found within the range of the species, and the species reaches higher altitudes here than elsewhere. In Monterey County, the northern extreme, the reasons for the limits of distribution are obscure, though winter temperatures are slightly lower than they are toward the center of distribution. It can only be said of this region that the existence of some limiting ecological factor is suggested by the fact that the species ends rather abruptly here (Pine Canyon, near King City).

The conditions postulated by Cleland (and by Darlington) for the high survival value of the complete ring include self-pollination, and preservation of heterosis by means of balanced lethals. In *Paeconia californica*, the difficulties of growing the plants (due chiefly to their being long-lived, slow-maturing perennials) have prevented experimentation to show whether there is a balanced-lethal mechanism at work. The presence of such a mechanism is suggested by the occurrence of homogeneous colonies such as the one, described above, at Altadena, but the contrary situation appears to exist in the heterogeneous colonies described. The regular occurrence of cross-rather than self-pollination is suggested by the fact that the colonies are morphologically heterogeneous; plants growing together show numerous individual differences, indicating a considerable degree of heterozygosity in addition to cross pollination. It is expected that evidence will soon be obtained showing definitely whether or not the plants are self-sterile. In any event, field observations of many hundreds of

plants have failed to show any greater degree of hybrid vigor in the ring-forming plants than in the pair-forming ones. Darlington and Gairdner (1937) have pointed out that self-sterility is an aid to the maintenance of interchange heterozygotes before the development of balanced lethals, and this suggestion may apply to *Paeconia californica* if the plants prove to be self-sterile.

The suggestion of Stebbins and Ellerton (1939), that interchanges which increase the size of the ring in *Paeconia* are probably caused by interlocking and subsequent breakage of chromosomes in the prophase of meiosis, seems to the author to be the most acceptable one. Interlocking is rather frequent in both pairs and rings (fig. 3), as also reported by Catcheside (1931) in *Oenothera* and by Sax (1931) in *Rhoeo*. That breakage may occur was also shown by Stebbins and Ellerton, and by Husted (1937). The occurrence of two-chromatid fragments at metaphase provides evidence of such breakage (fig. 5). One of the more striking things about meiosis in *Paeconia* is the relatively small size of the nucleus for the large size of the chromosomes; this fact, added to the marked tendency for pairing to occur only in the terminal portions of the chromosomes in both pairs and rings (Stebbins and Ellerton, 1939), may account for the numerous interchanges. Whether a balanced-lethal mechanism is at work, which tends to stabilize the rings in some cases, is as yet uncertain. The survival of interchange heterozygotes in the species in general is probably due, not to any particular survival value of the structurally heterozygous condition, but merely to the fact that interchanges occur frequently and to the added fact that in a long-lived perennial like *Paeconia*, fertility may be very greatly reduced without significantly affecting the reproductive ability of the species. A vigorous plant may bear a dozen or more flowers with over a hundred anthers per flower; thus the supply of pollen is large even with fertility reduced to 30 per cent, which is the approximate pollen fertility reported by Stebbins and Ellerton, and represents the lowest figure yet found by the author. The limiting factor in seed setting appears to be not ovule fertility, but the small size of the ovary for the size of the seeds; many of the developing seeds are regularly crowded out. In all field observations to date, there appears to be abundant seed set in both pair-forming and ring-forming plants.

Thus, it appears to the author that ring-formation in *Paeconia* does not have the positive selective value that has been adduced for it in *Oenothera*. Its probable causes may be summarized as follows: segmental interchange is favored by the large chromosomes, small nucleus, and terminally localized pairing, a combination of circumstances which favors interlocking and subsequent breakage. The structural hybrids which arise are not selected against, because in a long-lived perennial fertility may be much reduced and still leave ample reproductive ability. Therefore, through the ages, the structural hybridity has gradually accumulated.

SUMMARY

A study of structural hybrids in *Paeonia californica* ($n=5$) has revealed a complete series of interchange heterozygotes, including 5_{II} , $\odot 4$, $\odot 6$, $2 \odot 4$, $\odot 8$, $\odot 6 + \odot 4$, and $\odot 10$. This is the second complete series now known, paralleling the situation in *Oenothera* subg. *Onagra*. In addition, recognition of two of the five pairs in meiosis has made it possible to show differences in the constitution of rings of a given size, and to show alterations in chromosome morphology through unequal translocation. It is shown that there are certain regularities in the geographic distribution of the various types: a tendency is noted for pair-forming types and small rings to be more abundant near the center of the range of the species, and for the larger rings to

be more numerous near the periphery. Tentative suggestions are advanced to explain these regularities, and comparisons are made between the distributional features of *P. californica* and those of *O. iagra*. It is suggested from cytological and distributional evidence that there is no particular survival value associated with the complete ring in *Paeonia*, in contradistinction to *Onagra*, but that the extensive structural hybridity found is due to a combination of cytological circumstances favoring frequent interchange, added to the fact that the plants are long-lived perennials in which even large reduction of fertility does not significantly reduce reproductive ability.

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THE MORPHOLOGY OF *RIELLA AFFINIS*. II. DEVELOPMENT OF THE SEX ORGANS, FERTILIZATION, AND DEVELOPMENT OF THE SPOROPHYTE ¹

R. H. Thompson

IN A previous account of the investigation of *Riella affinis* (Thompson, 1941) vegetative development was described. The present paper completes the account of that investigation with the description of sexual development and development of the sporophyte of *Riella affinis*.

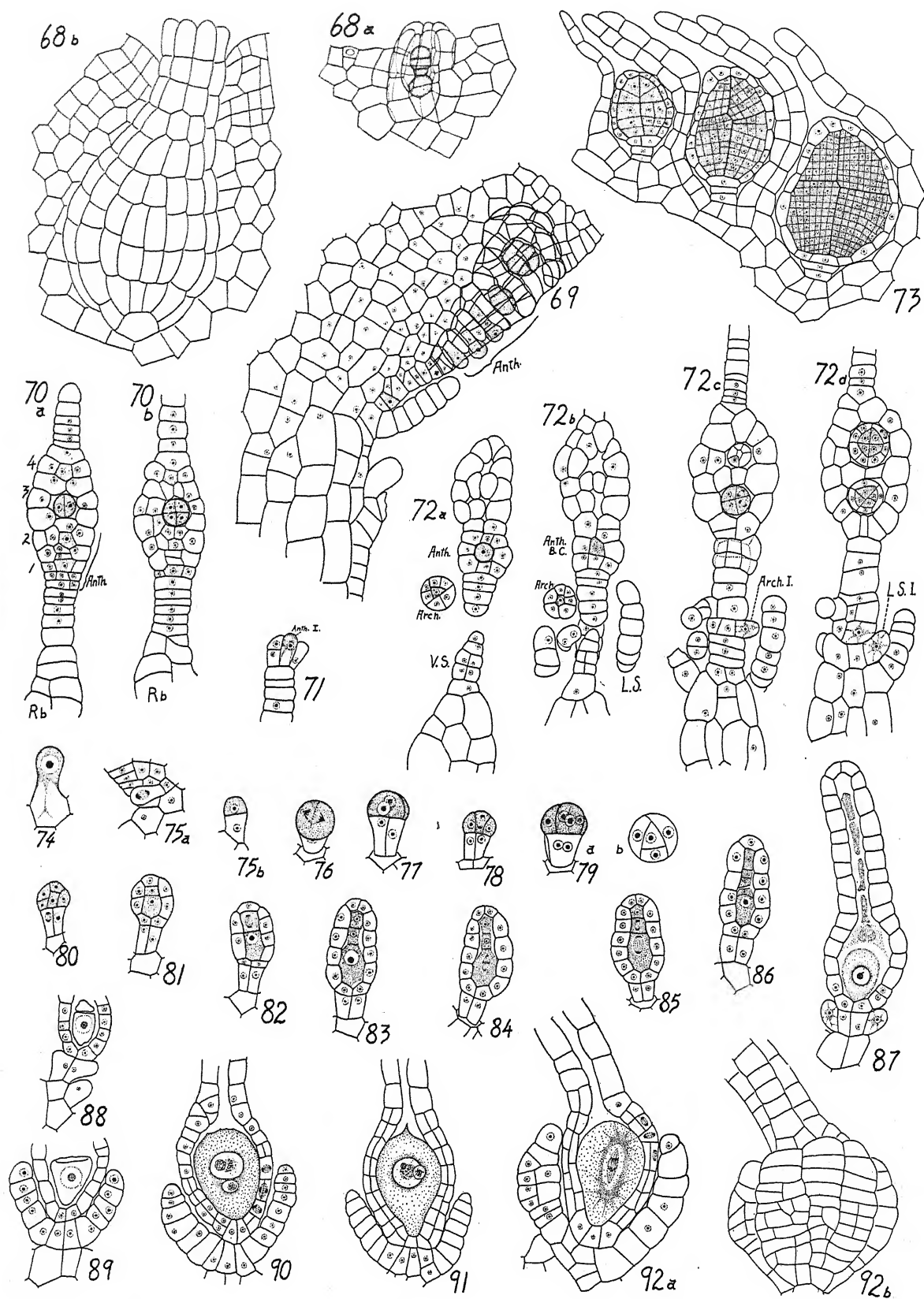
DEVELOPMENT OF SEX ORGANS: THE ANTHERIDIUM.—*R. affinis* is homothallic and protandrous. The first antheridia are produced very shortly after the apical cell is established. The mature antheridia lie in notches at irregular intervals along the margin of the wing. The base of the notch, the receptacle, sometimes bears a single antheridium but more fre-

quently two to four antheridia. Each antheridium is surrounded by a flask-shaped involucre (fig. 68a and 68b).

Hofmeister (1854), who was the first to describe antheridial development in *Riella*, held that a marginal cell of the wing produces a vesicular swelling beyond the edge of the wing and that the vesicular portion is cut off at the margin level. This antheridial initial is immediately surrounded by a cellular sheath produced by rapid growth of the cells adjacent to its base. Leitgeb (1879) investigated the same species of *Riella* as Hofmeister, but had only older stages in development of the antheridia. From a study of these he postulated that the antheridial initial and the involucre initials must have been produced simultaneously, and that this came about by two successive divisions of a marginal cell in the plane of the wing. Vrabner (1933) holds that one entire marginal cell of

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the wing functions as an antheridial initial. The initial divides unequally to form an inner basal cell and an outer cell. Two successive longitudinal divisions of the basal cell, in the plane of the wing, produce a single involucre initial on each side of, and below, the developing antheridium. The antheridial involucre is produced by these two initials together with the two adjacent marginal wing cells. The following account of antheridial development in *R. affinis* confirms Leitgeb's deduction concerning the early stages of development.

The antheridial initial and the primary cells of the involucre are formed from a group of three adjacent marginal cells two or three times removed from the apical cell (fig. 69, Anth.). Each of the three cells divides unequally in the plane of the wing. The larger of the two daughter cells formed by each division then divides in the same manner (fig. 70a, Anth.). This produces a group of nine cells of which the central one is the antheridial initial and the surrounding eight cells are the initial cells of the involucre (fig. 71, 72a).

The antheridial initial enlarges at its outer free end and protrudes slightly above the level of the adjoining involucre initials (fig. 69). It then divides transversely. This division is unequal and produces a rectangular inner cell, the basal cell, and a globose outer cell (fig. 69). Two successive transverse divisions of the outer cell produce a young antheridium which is composed of three superimposed cells, and which is ovoid in shape (fig. 68a, 69). The innermost cell of these three divides transversely to produce the two-celled stalk of the mature antheridium. The two remaining cells produce the capsular portion of the mature antheridium. Each of the two cells, developing into the capsular portion, divides longitudinally and generally in the plane of the wing (fig. 70a, Anth. 3). As a rule the cell nearest the stalk divides first, and further division in this tier precedes that in the outer tier (fig. 70a and b, Anth. 3). Next, each cell in each of the two tiers divides longitudinally but in a plane at right angles to that of the first division (fig. 70b). Each cell of the quadrants thus formed then divides periclinally (fig. 72c and d). These divisions produce eight peripheral cells, which are the primary jacket cells of the antheridium, and eight central cells, which are the primary androgonial cells. The primary androgonial cells enlarge and divide in three planes. Repeated division of the androgonial cells produces a large number of androcyte mother cells (fig. 73). As is the case in most Hepaticae, divisions are simultaneous in each group of

cells derived from a single primary androgonial cell (fig. 73). In the meantime the primary jacket cells divide in two planes so that the growth of the antheridial jacket keeps pace with that of the interior. Chloroplasts appear in the jacket cells very early, but they never become as prominent as those in the involucre and wing cells.

Growth of the involucre is simultaneous with that of the antheridium. The eight involucre initials surrounding the antheridial initial function together as a ring of apical initials. The ring of apical initials successively cuts off seven or eight tiers of derivatives. Each cell in the median tiers produces several daughter cells by longitudinal division. Longitudinal divisions are fewer in the last formed tiers. When the eight apical initials cease dividing they elongate greatly to form the neck of the now flask-shaped involucre (fig. 69b).

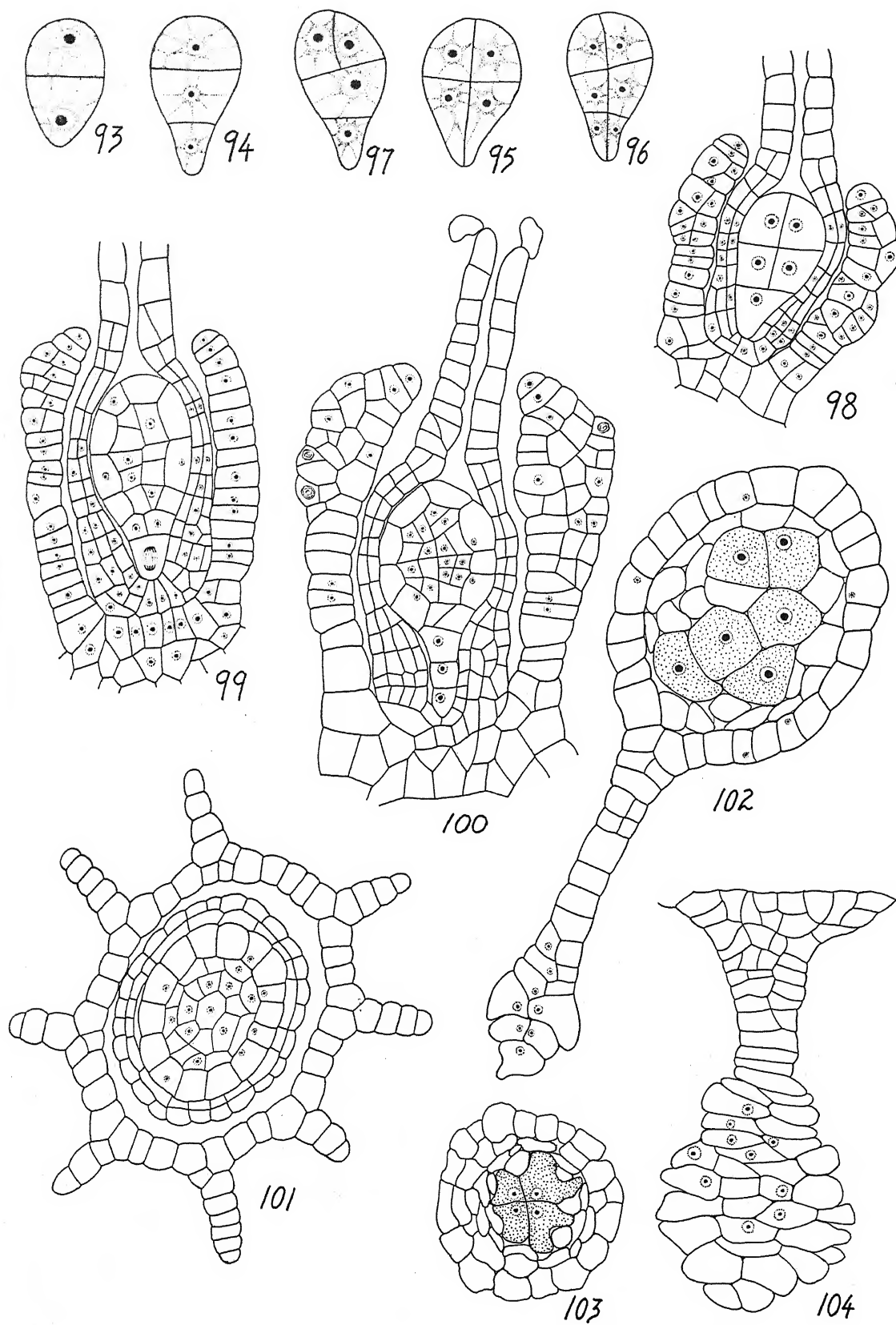
During maturation of the involucre cells the chloroplasts in the jacket cells of the antheridium become orange-red. At the same time the walls of the androcyte mother cells begin to gelatinize. Breakdown of the walls continues during division of the androcyte mother cells to form androcytes and during the metamorphosis of these into antherozoids. The coiled antherozoids soon lie free in the viscous fluid within the capsule of the jacket.

Explosive discharge of the antherozoids was observed both in culture and in temporary mounts. This phenomenon is unusual inasmuch as it has only been reported in the case of *Asterella*, *Conocephalum*, *Marchantia*, and *R. helicophylla*. More frequently, however, the antherozoids are exuded in a slow stream through the ruptured apex of the antheridial jacket. They may become active while still within the antheridium or only after they are released.

THE ARCHEGONIUM.—Archegonia are borne on both sides of the adult thallus on the morphologically dorsal side of the rib. In *R. affinis* they are produced in acropetalous succession, generally in series of three to five, and generally during periods alternate with those of antheridial formation in the wing.

An archegonial initial is a wing cell which is generally twice removed from the apical cell and lies in the region of juncture of wing and rib (fig. 32-34, 74, 75). This initial forms a cylindrical protrusion which becomes enlarged and broadly rounded at its tip. Chloroplasts are not evident in the protrusion, and the protoplasm in the apical portion is densely granular. The initial divides transversely, and the wall formed is at the level of the thallus surface. Of

Fig. 68-92, $\times 285$.—Fig. 68a, 68b. Young and mature antheridial involucres.—Fig. 69. Antheridial initiation and early development (three-dimensional drawing from a whole mount).—Fig. 70a, 70b. Consecutive cross sections of the wing in the region of antheridial development. Anth., antheridium; Rb, rib; Wg, wing.—Fig. 71. Cross section of the wing and longitudinal section of an antheridial initial; two involucre initials, one of which has divided to produce an apical cell.—Fig. 72a-72d. Serial cross sections in region of antheridial development. V.S., ventral scale; L.S., lateral scale; L.S.I., lateral scale initial; Arch., archegonium; Arch. I., archegonium initial; Anth. B.C., antheridial basal cell; Anth. I., antheridial initial.—Fig. 73. Tangential section of the wing and longitudinal section of older antheridia showing from left to right successively older stages in development.—Fig. 74-87. Stages in development of the archegonium.—Fig. 87-92b. Stages in the development of the archegonial involucre.—Fig. 90, 91. Two phases of fertilization.—Fig. 92. First division of the zygote.



the two daughter cells formed by this division, the short basal cell remains embedded in the tissue at the base of the wing, and the outer cell stands above the thallus surface. The outer cell divides transversely, but unequally, to produce two daughter cells (fig. 75*a* and *b*). The inner, larger daughter cell, which is adjacent to the basal cell, is the primary stalk cell of the archegonium. The sister cell develops into the flask-shaped portion of the archegonium.

The cell producing the flask-shaped portion of the archegonium enlarges and divides longitudinally into two daughter cells of unequal size (fig. 76, 77). The larger of the two daughter cells divides longitudinally and unequally but in a plane anticlinal to that of the first division. The larger of the two cells formed by this division then divides periclinally, so that there is produced a primary axial cell and three peripheral cells (fig. 79*a* and *b*). A longitudinal division of each peripheral cell results in a ring of six jacket initials around the primary axial cell (fig. 72*a* and 72*b*, Arch.). During the longitudinal division of the peripheral cells the primary axial cell also divides, but transversely and unequally. The upper and smaller daughter cell of this division is the primary cover cell (fig. 78). The lower and larger daughter cell is the central cell of the archegonium. In the meantime the primary stalk cell has divided longitudinally and generally in a plane at right angles to that of the first division in its sister cell above (fig. 77).

There is next a nearly simultaneous transverse division of each of the six jacket initials (fig. 80, 81). Each of these divisions is unequal and produces two daughter cells of which the upper one is the smaller. The six upper daughter cells formed by this division of the jacket initials are the neck initials. The six lower daughter cells are the venter initials. Coincident with division of the jacket initials there is an unequal, transverse division of the central cell. The upper daughter cell is the primary canal cell and the lower daughter cell is the primary ventral cell (fig. 81). The nucleus of the latter cell is very large and remains close to the newly formed wall.

During the formation of venter and neck initials each of the two stalk cells divides longitudinally and in a plane at right angles to the wall between the two. The four stalk cells thus formed now divide transversely and unequally (fig. 80, 81). The four upper daughter cells, which are the smaller, constitute the floor of the venter of the archegonium. The four lower daughter cells constitute the archegonial stalk and later produce the archegonial involucre.

The six venter initials divide in two planes and produce the venter of the archegonium. Division of the six neck initials is transverse and produces the neck of the archegonium.

During the development of the venter and neck of the archegonium, the divisions of the primary canal cell and primary ventral cell are completed. The pri-

mary canal cell divides transversely to form two daughter cells (fig. 82-84). Each of these, generally the inner of the two first, also divides transversely, with the result that four neck canal cells are formed (fig. 85, 86). During the divisions that produce the neck canal cells, the primary ventral cell divides transversely and unequally to produce two daughter cells, of which the lower and larger one is the egg cell and the upper and smaller one is the ventral canal cell (fig. 84-86).

The archegonium grows rapidly in size after the completion of the division to form ventral canal cell and egg. During this growth the primary cover cell divides quadrately to form four cover cells at the apex of the archegonium.

Each of the neck canal cells and the ventral canal cell shrinks away from the walls of the neck cells and gradually disintegrates. The egg cell swells considerably and a large amount of the cytoplasm becomes aggregated at the periphery of the nuclear membrane (fig. 87). The nucleus of the egg is very large and contains a dense central body surrounded by a wide hyaline area between it and the nuclear membrane. At maturity of the archegonium the four cover cells swell and separate from each other, leaving the neck of the archegonium open for the entrance of antherozoids.

The mature archegonial involucre of *R. affinis* is ovoid and ornamented with eight wing-like, longitudinal ribs. Of the seventeen species of *Riella*, *R. affinis* is one of the three with such ribs on the involucre.

Vraber (1933) describes in *R. helicophylla* the formation of a massive archegonial stalk. The peripheral cells of the stalk adjacent to the venter of the archegonium function as involucre initials.

In *R. affinis* the archegonial involucre originates from the four stalk cells of the archegonium. At about the time of the formation of neck canal cells, the stalk cells begin to bulge out at the upper end of each cell (fig. 88). Each stalk cell then divides longitudinally to produce two daughter cells, the outer of which is an involucre initial (fig. 87). Each of the four involucre initials functions as an apical cell and successively cuts off three or four derivatives (fig. 89). All but the lowermost of the derivatives divide once in a longitudinal plane. If the egg in the archegonium is not fertilized, there is no further development of the involucre; if the egg is fertilized, each of the four apical cells divides longitudinally to form two apical cells. Each cell in the ring of eight apical cells cuts off several derivatives. A derivative once removed from its apical cell divides longitudinally in a plane parallel to the surface of the involucre. The outer daughter cell by division and redivision contributes to the rib or wing portion of the involucre (fig. 92*a* and *b*, 98-100). Since there are eight longitudinal rows of derivatives, there are generally eight involucre ribs, each a sheet one cell in thickness (fig.

Fig. 93-104, $\times 363$. Sporophyte development.—Fig. 98-101. Development of the involucre.—Fig. 101. Cross section of a young sporophyte and its involucre.—Fig. 103. Cross section of a young sporophyte foot of about the same age as figure 102.—Fig. 104. Fully developed foot.

101). The inner daughter cell, formed by periclinal division of a derivative, by division and redivision in two planes contributes to the body of the involucre. About the time the sporophyte is forming spore mother cells, each apical cell elongates greatly and matures into a papillate terminal cell at the apex of the involucre.

FERTILIZATION.—Fertilization has been observed several times, but all the cases fall into two phases of gametic union. Each of these can be correlated with similar phases in *R. helicophylla* described by Kruch (1890). Each can be compared more exactly with phases described by Rickett (1923) for *Sphaerocarpos Donnellii* Austin. As is the case in *S. Donnellii*, the position of the male nucleus in the egg of *R. affinis* is variable (fig. 90, 91). The earlier of the two phases observed (fig. 90) is similar to that shown in Rickett's figure 4, which he termed the third phase of fertilization. In this phase there is no evidence of chromosome formation. The later phase of gametic union found in *R. affinis* (fig. 91) is similar to Rickett's sixth phase in which the chromosomes have become evident in the male nucleus.

As is the case with other Hepaticae the cells of the venter begin periclinal division shortly after the antherozoid penetrates the egg. Periclinal division continues during the phases leading up to fusion of the gamete nuclei so that by the time of the first division of the zygote the venter is two cells thick throughout (fig. 90–92a).

DEVELOPMENT OF THE SPOROPHYTE.—The mature sporophyte of *Ricella* consists of a globose capsule, a short seta and a bulbous foot. The jacket of the capsule, which is one cell thick, encloses two kinds of cells, spores and nurse cells. The spores at first lie in tetrads, but at maturity lie entirely free from one another.

Hofmeister (1854) states that the first division of the zygote of *R. Reuteri* is transverse and that both daughter cells divide transversely. The two upper cells in the filament of four produce the capsule of a mature sporophyte, and the two lower cells produce the seta and foot. Vrabner (1933) also reports a similar development of the sporophyte in *R. helicophylla*. The first division of the zygote in *R. affinis* is transverse (fig. 92a, 93). Usually there is an elongation of the hypobasal cell and then a transverse division of this cell (fig. 94). Of the filament of three cells thus formed the two upper cells produce the capsular portion of the mature sporophyte and the lowermost produces the seta and foot portion.

In some cases after transverse division of the zygote there is a longitudinal division of both epibasal and hypobasal cells to form a four-celled embryo with quadrately disposed cells (fig. 95). The pair of hypobasal cells then divides transversely (fig. 96). In these six-celled embryos the four upper cells produce the capsule of the mature sporophyte, the two lower cells produce the seta and foot.

If the young embryo is a three-celled filament the two upper cells divide longitudinally, and each

daughter cell also divides longitudinally but in a plane at right angles to that of the preceding division (fig. 97, 98). The embryonic capsule now consists of two superimposed quadrants. Next, each cell of a quadrant divides periclinally to form an outer primary jacket cell and an inner primary archesporial cell. The primary archesporial cells divide successively in three planes to produce a large number of sporogenous cells (fig. 99–102). The primary jacket cells divide successively in two planes and in a very regular sequence. Some of the sporogenous cells enlarge to form spore mother cells each of which then produces a tetrad of spores. The remaining sporogenous cells develop chlorophyll and accumulate starch. These are termed nurse cells and as in the case of those in *Sphaerocarpos Donnellii* (Gross and Allen, 1938), they are four-nucleate.

During the first few divisions of the primary archesporial cells the lowermost cell of the embryo sporophyte divides transversely and successively to form a linear series of four cells (fig. 99, 100). Successive transverse division of each of these increases the number of cells to fifteen or twenty (fig. 102). Further division of the upper five or six cells is in three planes, so that the upper end of the seta becomes broad. Further division of the lower six to ten cells is also in three planes and produces the globose, multicellular foot (fig. 102–104). In between these two regions, the cells divide once or twice longitudinally. The cells of the developing foot enlarge laterally, forming finger-like projections that become insinuated between the cells of the gametophyte tissue (fig. 104).

During the growth of the sporophyte, periclinal division continues in the portion of the venter which surrounds the seta and foot. This portion of the venter becomes three to six cells thick (fig. 98–100). The basal cell of the archegonium and the stalk cells also divide many times (fig. 99). Division in three planes of the basal cell and the adjacent wing and rib cells produces a short stalk that elevates the sporophyte and its involucre above the level of the rib surface.

SUMMARY

The account of the development of the antheridium and the antheridial involucre in *R. affinis* confirms Leitgeb's deduction that these two structures are formed simultaneously.

The development of the archegonium parallels that found in the Marchantiales. The stalk cells not only give rise to the floor of the venter but also produce the initials from which the archegonial involucre develops.

The development of the sporophyte is the same as that in *Sphaerocarpos*, and, as is true in *S. Donnellii*, the nurse cells are four-nucleate.

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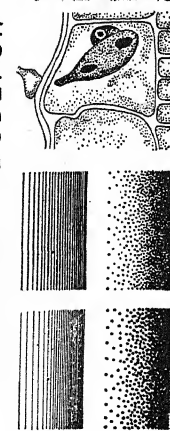
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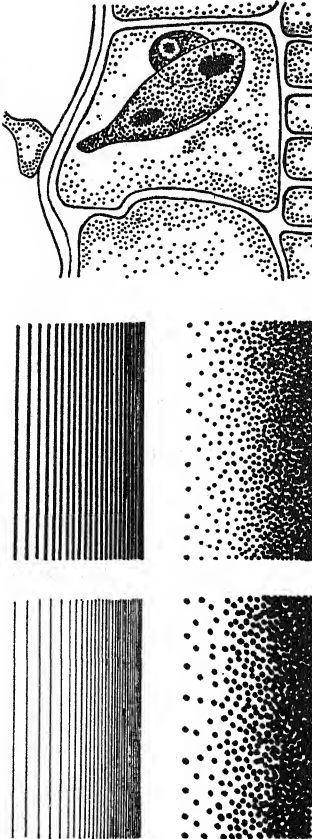
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DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

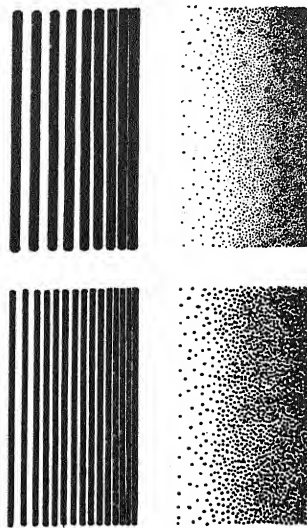
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Note that thin black lines hold up fairly well in reduction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. Keep the shading rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

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POLYPLOIDY IN *SEDUM TERNATUM* MICHX. II. CYTOGEOGRAPHY¹

J. T. Baldwin, Jr.

THIS PAPER gives the geographic occurrence of four chromosome-number races of *Sedum ternatum* Michx. as revealed by routine cytological analysis of the species in the wild. It is one of a series of reports planned on the cytogeography² of North American plants: those on *Galax* L. (Baldwin, 1941), on *Diospyros virginiana* L. (Baldwin and Culp, 1941), and on *Oxydendrum* DC. (Baldwin, 1942) have been written; those on *Leavenworthia* Torr. and on *Sedum pulchellum* Michx. are in preparation. Cytological survey of many species throughout their geographic and ecologic ranges should throw light on such problems as frequency of intraspecific chromosome races; concentration, or lack of concentration, of such races in certain regions and situations; significance of polyploidy; dispersal paths of species; trends in evolution of floras; systematic and phyletic relationships, etc.

Plants of *S. ternatum* were collected from the wild either by the writer or by co-operating individuals (see table 1) and, in most cases, grown in pots at the University of Michigan Botanical Gardens. The chromosomes were usually counted in aceto-carminic smears of roots after fixation in Carnoy's fluid. Such preparations of this species are easily made. Nawa-schin-fixed, crystal-violet-stained sections of roots from some of the collections were studied.

Chromosome numbers were determined for seventy-one collections from locations throughout the

range of the species (table 1 and map 1): eleven collections were diploid— $2n=16$ (fig. 1); two, triploid³— $2n=24$ (fig. 2); fifty-seven, tetraploid— $2n=32$ (fig. 3); one, hexaploid— $2n=48$ (fig. 4). Diploid and tetraploid plants have been previously reported in this species (Baldwin, 1936).

Figure 5 shows the distribution of *S. ternatum* as known to the writer by collections investigated cytologically or by specimens examined by him in certain herbaria.⁴ The map reveals various things about the geography of the races within this species: the diploid has been found in a relatively restricted area in southern West Virginia, southwestern Virginia, and eastern Kentucky; the tetraploid radiates from that area throughout the range of the species—to New York, Michigan, Indiana, Tennessee, Alabama, and North Carolina; the triploid was collected in West Virginia in the region where both the diploid and tetraploid occur and in North Carolina where only the tetraploid is known, but where the diploid might be expected to occur; and the hexaploid was discovered at only one station—near Tuscaloosa, Alabama, approximately the southern limit of the species, and in the vicinity of the tetraploid. Presumably the diploid is primitive, and the other races are derived. The area of the diploid is accordingly the center from which the species has spread in perimetric fashion. It is logical to conclude that the several races are not isolated genetically: existence in nature of the trip-

³ The somatic number of the triploid may be designated as $2n$ or $3x$; of the tetraploid, as $2n$ or $4x$, etc.

⁴ United States National Herbarium, Gray Herbarium, herbaria of the New York Botanical Garden, of the Missouri Botanical Garden, of Cornell University, Duke University, West Virginia University, University of Georgia, University of Kentucky, University of Michigan, University of Tennessee, and private herbarium of Charles C. Deam. Appreciation is here expressed for the privilege of studying these specimens.

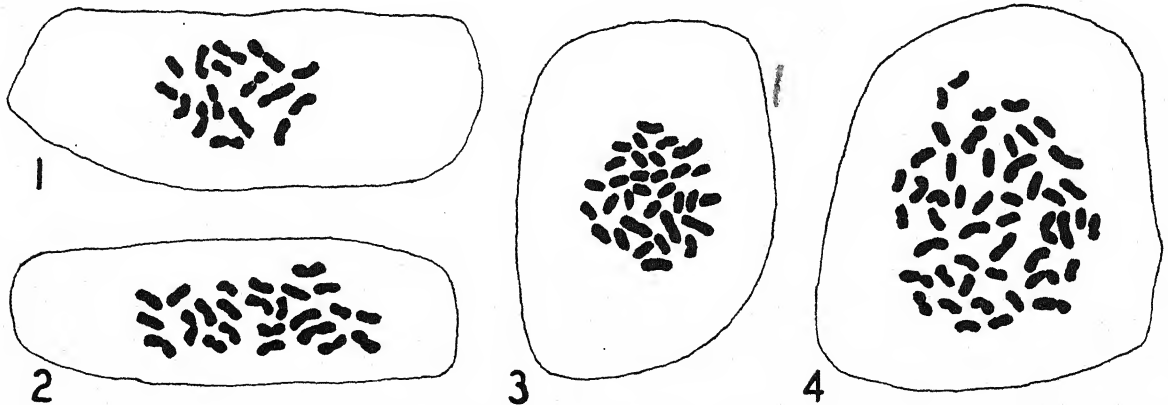


Fig. 1-4. Cells with metaphase chromosomes as drawn from root-tip smears of *S. ternatum*: they show a polyploid series of 16, 24, 32, and 48. Ca. 2,000 \times .

[The Journal for March (29: 195-281) was issued April 2, 1942.]
AMERICAN JOURNAL OF BOTANY, VOL. 29, No. 4, APRIL, 1942.

TABLE 1. *Collections of Sedum ternatum studied cytologically.*

Locality	2n-number of chromo- somes	Collector	Locality	2n-number of chromo- somes	Collector
Alabama:			Madison County:		
Bibb County:			Mars Hill	32	B.
Pratt's Ferry Bridge	32	B. ^a R. M. Harper, and A. V. Beatty	McDowell County:		
Tuscaloosa County:			Linnville Mountain.	24	B.
Warrior River	32	A. V. Beatty	Woodlawn	32	B.
Warrior River	48	B., R. M. Harper, and A. V. Beatty	Mitchell County:		
Indiana:			Spruce Pine	32	B.
Grant County:			Watauga County:		
Sims	32	R. C. Friesner	Boone	32	B.
Huntington County:			Yancey County:		
Markle	32	P. H. Cook	Burnsville	32	B.
Wabash County:			Ohio:		
Lagro	32	P. H. Cook	Butler County:		
Warren County	32	A. T. Guard	Seven Mile	32	B.
Kentucky:			Hamilton County:		
Anderson County:			Dunlap	32	B.
Lawrenceburg	32	B., S., and H. ^b	Hocking County:		
Casey County:			Logan	32	R. A. Popham
Dunnville	32	B., S., and H.	Preble County:		
Liberty	32	B., S., and H.	Eaton	32	B.
Clark County:			South of Eaton . . .	32	B.
Lower Howard			Pennsylvania:		
Creek	32	B., S., and H.	Delaware County:		
Clinton County:			Darby Creek	32	Anonymous
Albany	32	B., S., and H.	Lancaster County:		
Fayette County:			Chickies	32	Anonymous
Raven Creek	32	B., S., and H.	Tennessee:		
Harlan County:			Knox County:		
Lynch	16	B.	Holston River	32	B.
Hart County:			Overton County:		
Mumfordsville	32	B., S., and H.	Carr Creek	32	B., S., and H.
Lincoln County:			Pickett County:		
Hustonville	32	B., S., and H.	Wirmingham	32	B., S., and H.
Meade County:			Putnam County:		
Buttermilk Falls . .	32	B., S., and H.	Monterey	32	B., and W. C. Gregory
Grahamtown	32	B., S., and H.	Scott County	32	B.
Nelson County:			Smith County:		
Cedar Creek	32	B., S., and H.	Chestnut Mound . . .	32	B., S., and H.
Russell County:			Sumner County:		
Rowena	32	B., S., and H.	Near Goodlettsville.	32	B., S., and H.
Whiteley County:			White House	32	B., S., and H.
Cumberland Falls . .	32	B.	West Virginia:		
Woodford County:			Braxton County	32	B.
Clifton	32	B., S., and H.	Cabell County	32	F. A. Gilbert
Michigan:			Greenbrier County:		
Washtenaw County:			Lewisburg	16	W. S. Flory
Ann Arbor	32	B.	Lewisburg	24	B., and H. B. Graybill
New York:			Mercer County	16	B.
Tompkins County:			Monongalia County:		
Ithaca	32	B.	Morgantown	32	Mary L. Grumbein
Ithaca (three collec- tions)	32	A. Schulze	Monroe County:		
North Carolina:			Wolf Creek	16	B.
Buncombe County:			Nicholas County:		
Asheville	32	B.	Summersville	32	B.
Jackson County:			Pocahontas County:		
Sylva	32	B.	Buckeye	32	B.
			Wyoming County:		
			Mullens	16	B.
			Wolf Pen	16	B.
			Virginia:		
			Clarke County:		
			White Post	32	B.

^a J. T. Baldwin, Jr.^b J. T. Baldwin, Jr., H. T. Shacklette, and A. M. Harvill.

TABLE 1. *Concluded.*

Locality	$2n$ -number of chromo- somes	Collector
Fairfax County:		
Great Falls	32	O. E. White
Giles County:		
Mountain Lake	16	G. B. Wolcott
Narrows	32	B.
Rich Creek	16	B.
Glen Lyn	16	B.
Roanoke County:		
Roanoke	32	Bernice M. Speese
Russell County:		
Lebanon	16	B.
Wise County:		
Appalachia	16	B.
Wythe County:		
Wytheville	32	H. M. Showalter

loid race and its physical proximity to both the diploid and tetraploid races, as well as the discontinuous distribution of the triploid, support this conclusion. All the races maintain themselves vegetatively; it has not yet been determined whether or not they all produce good seed.

There can be no question but that these races belong to a single species: they quite obviously do. As pointed out by Wherry (1935), this is the only species of *Sedum* in the eastern United States "concerning which no taxonomic difficulties have arisen, its broad obovate leaves consistently whorled in threes being highly distinctive." The different collections, under uniform conditions in the greenhouse, vary considerably in appearance. Yet the four chromosome-number races are not readily separable except by cytological study; an attempt will be made to distinguish them by statistical measurement. However, during the summer of 1941, most of the tetraploid plants and the triploid from Lewisburg, West Virginia, were more resistant than the other collections to the attack of a fungus of undetermined nature.

If glaciation has, in some cases, as perhaps in *S. ternatum*, induced polyploidy, two explanations for such influence may be suggested. One, temperature changes might so alter the viscosity of the protoplasm during nuclear division that the precise mechanism of the division would be upset. Two, glacial movement would continually create new ecological situations into which a species might migrate, and, there, representatives from different parts of the specific

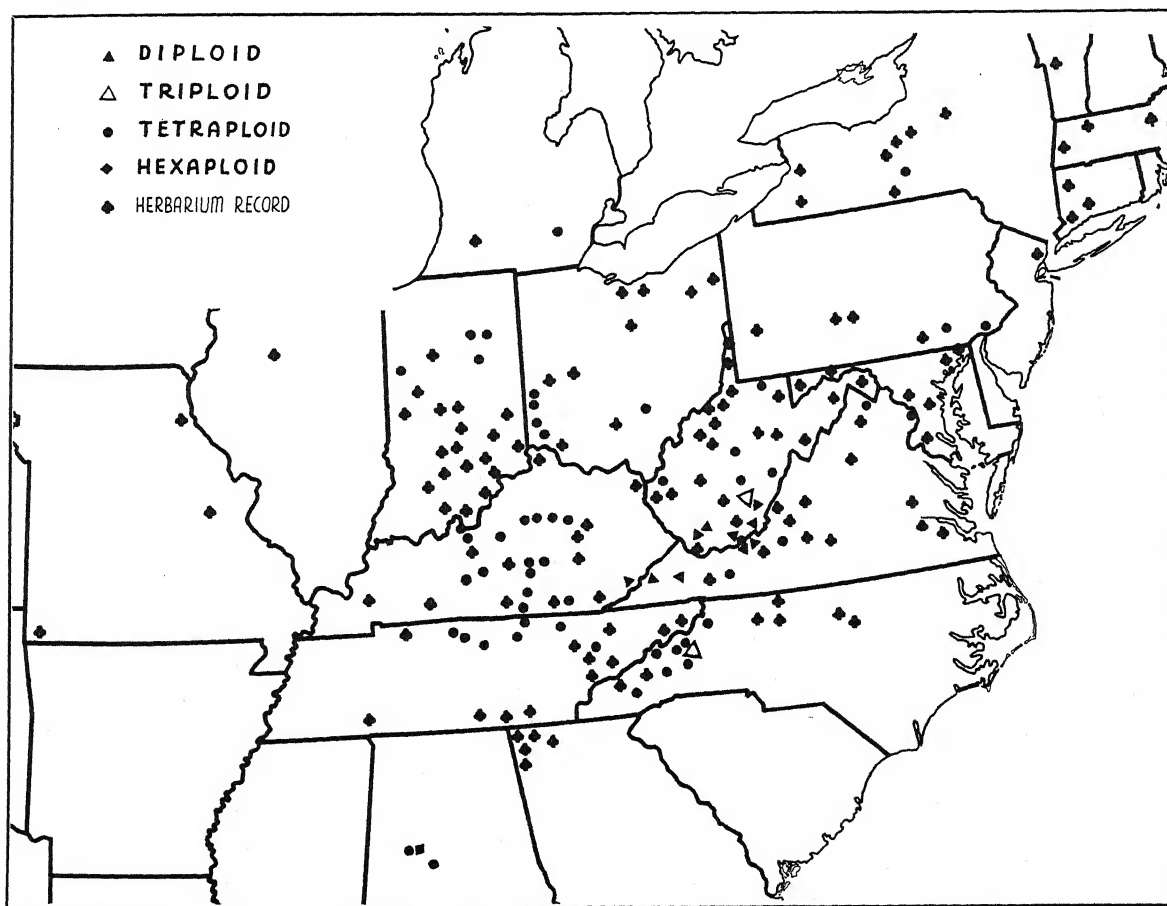


Fig. 5. Distribution of *S. ternatum* from Massachusetts to Missouri and Alabama, as based on herbarium specimens and on collections for cytological study.

range, with chromosomes differentially rearranged, might be brought together to produce polyploid individuals through hybridization.

SUMMARY

Four chromosome-number races of *S. ternatum* Michx. are known in the wild: $2n=16$ (diploid), $2n=24$ (triploid), $2n=32$ (tetraploid), and $2n=48$ (hexaploid).

Geography of the races was determined on the basis of cytological study of seventy-one collections of plants from representative points in the specific area (map 1 and table 1). The diploid, and supposedly primitive, race (eleven collections) was found in a restricted region in West Virginia, Kentucky, and Virginia. The tetraploid (fifty-seven collections) is widespread and radiates from the limited region of the diploid as a center. The triploid (two collections) seemingly has a discontinuous distribution; it was found in West Virginia near both the diploid and the tetraploid races and in North Carolina where both these other races may be expected. The hexaploid (one collection) was found near Tuscaloosa, Alabama, at the southern edge of the specific range and near the tetraploid race.

The several races are apparently not genetically isolated, indications being that the triploid arises through natural hybridization of diploid and tetraploid plants. All the races can maintain themselves vegetatively.

Specimens of *S. ternatum* from different localities often differ much, but it has not been possible, except by cytological means, to draw ready lines of separation between the chromosome-number races.

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ALTERATION OF POTATO STARCH GRAIN STRUCTURE UNDER THE INFLUENCE OF DISEASE¹

D. B. O. Savile

IN 1939, during the examination of potato tubers that showed rusty internal flecks seemingly caused by a virus, the identity of which is still in doubt, it was found that a high proportion of the starch grains in cells adjacent to the necrotic areas were distinctly abnormal in form. In view of its possible diagnostic value, the unusual appearance of these grains encouraged an investigation of their origin.

As a pathologist's tool the phenomenon has proved disappointing; for malformed grains have been detected in specimens of a variety of fungous, virous or environmental disorders, though never in the abundance that was seen in the original material. Furthermore, when this necrosis originated in storage the malformed grains were found to be far less plentiful than in specimens submitted at digging time. Botanically, however, these abnormal grains are of considerable interest, for they are evidently the visible results of abnormal function of the leucoplasts that lay them down.

It may be well to emphasize at the outset that these grains are not eroded by hydrolysis. Cell division and cork formation occur at the edges of the necrotic areas, as is frequently the case in injured tissue, and in the dividing cells there is, as always, starch hydrolysis; but the partly hydrolyzed grains, examples

of which are shown in figure 6, are pitted and tunneled precisely like similar grains in normal tubers. The malformed grains, illustrated in figures 1, 3, 4, and 5, are markedly different in appearance. It may be noted that the two small grains at the right in figure 6 are pointed at one end, and it is possible that, in addition to being partly hydrolyzed, they are slightly malformed.

It is not only the leucoplasts that behave abnormally. Nuclear divisions are frequent, but they are not always the regular divisions connected with phellogen formation. Often three, four, or even five nuclei are seen in a single cell. Figure 2 shows a small binucleate cell not connected with any phellogen. Owing to the considerable depth of focus required, it is seldom possible to show more than two nuclei in a photograph. Figure 4 is from a camera-lucida drawing of a pentanucleate cell. It will be seen that the nuclei differ considerably in size, their appearance suggesting the result of fragmentation rather than typical mitosis.

It should be noted that the grains close to the nuclei in figures 1 and 4 (i.e., those last formed) are particularly irregular, whereas those further away are usually normal. This distribution was found to be general and indicates a progressive degeneration within the cell.

An examination of the moderately abnormal grains, many of which resemble in form nothing so much as dipterous larvae (fig. 5, b and c), indicates

¹ Received for publication November 12, 1941.

Contribution No. 684 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

the way in which they are formed. The grains shown in figure 5 were drawn under crossed nicols in order to show clearly the position of the hilum. The leucoplast appears to become increasingly degenerate during the formation of the grain, for the hilum is close to the more normal end. It would seem that, under the influence of some toxic material (possibly in this case the virus, but probably sometimes a degradation product from adjacent dead cells), the leucoplast either loses much of its elasticity or suffers from

same periodic deposition that is observable in normal grains, but it is possible that they represent merely a spasmodic withdrawal of the degenerating leucoplast.

The abundance of abnormal grains in tubers showing necrosis at harvest time and their scarcity in tubers that became necrotic during the winter are further evidence, should any be required, that these grains are truly malformed and are not normal grains that have been eroded in some novel manner. However, small numbers of such grains have been seen, for example, in tubers invaded by the late blight fungus (*Phytophthora infestans*). In such material it is almost certain that the tubers were still normal when starch formation ceased with the death of the plant. It, therefore, seems probable that when starch

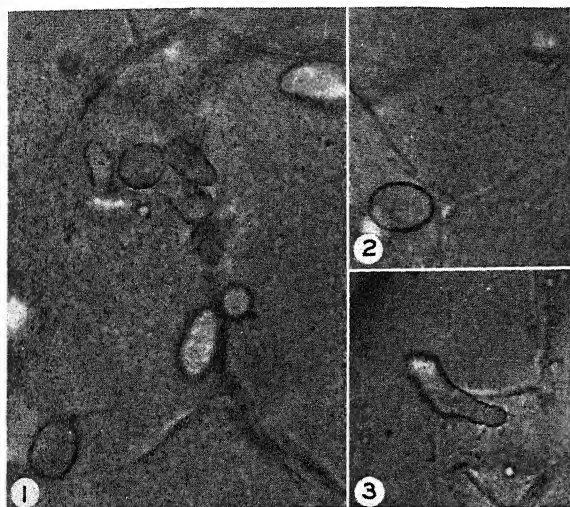


Fig. 1-3. All figures from unstained freehand sections and approximately $\times 600$.—Fig. 1. Parenchyma cell, showing several malformed grains close to the nucleus.—Fig. 2. Small binucleate cell, not part of a phellogen.—Fig. 3. Severely malformed grain.

some surface tension effect. The result of the change, whatever its nature, is the rupturing of the leucoplast on the thin side, where the hilum is closest to the surface of the grain. Once ruptured, the leucoplast withdraws to the opposite side of the grain. Succeeding starch deposition takes the form of caps, one upon another, at this part of the grain.

Sometimes the plastid remains normal until late in the formation of the grain and then degenerates abruptly as at figure 5a; at others degeneration proceeds more gradually with the result that several successive depositions are of nearly equal size (fig. 5b). Severely affected grains seem often to be the products of leucoplasts that are abnormal from the start of deposition. Such grains, which are often pointed at both ends like that in figure 3 and that marked "x" in figure 4, show no dark cross under crossed nicols; but it is seen, from the passage of the dark bands across them when they are centered and the stage is rotated, that the hilum is terminal or virtually so. The extremely small abnormal grains have no readily observable structure, but they, like the larger ones, stain quite as deeply with iodine as do the normal grains.

It seems probable that the "segments" that give the grains their larva-like appearance are due to the

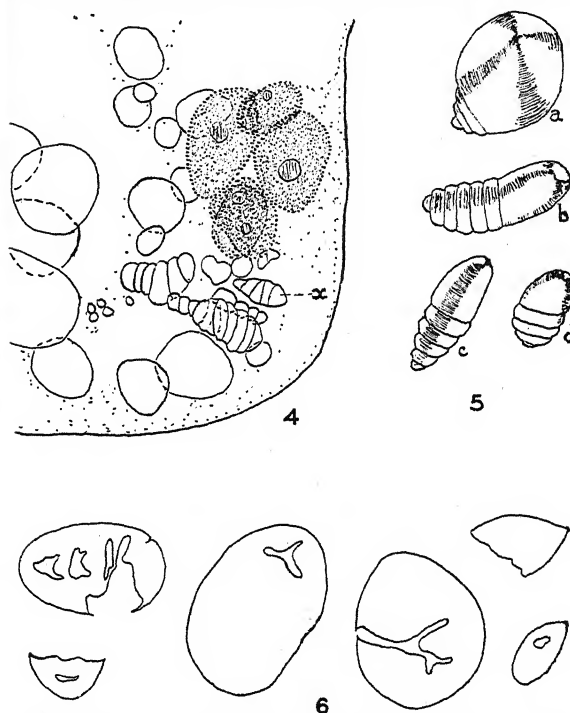


Fig. 4-6. Camera-lucida drawings, approximately $\times 600$.—Fig. 4. Part of pentanucleate cell with malformed grains close to the nuclei.—Fig. 5. Malformed grains viewed under crossed nicols, showing position of hilum near the most normal side.—Fig. 6. Partly hydrolyzed grains, in cells near phellogen, for comparison with malformed grains.

is hydrolyzed, as in the formation of a phellogen, some of the resulting sugar is not used as a source of energy but is transported to other cells and re-formed into starch.

SUMMARY

The occurrence of malformed starch grains in diseased potato tubers is described; it is suggested that their formation is attributable to an altered physical state of the leucoplasts. Associated nuclear abnormalities are also described.

CENTRAL EXPERIMENTAL FARM,
OTTAWA, CANADA

STRUCTURE OF THE APICAL MERISTEM AND DEVELOPMENT OF THE FOLIAGE LEAVES OF *CUNNINGHAMIA LANCEOLATA*¹

G. L. Cross

THE PRESENT paper is the fourth in a series dealing with the structure and development of the shoot apices and foliage leaves in the genera of the Taxodiaceae. The results obtained thus far (Cross, 1939, 1940, 1941) indicate that comprehensive information concerning the various genera in this family may be of aid in understanding the evolutionary processes

per cent commercial formalin and 6 per cent glacial acetic acid, made up in 70 per cent ethyl alcohol. Tertiary butyl alcohol was used for dehydrating and clearing, and the materials were embedded in "Tissue Mat." Sections were cut 8–10 μ in thickness at 65° F., and were stained with safranin and fast green (Cross, 1937). The illustrations were prepared as described in previous papers (Cross, 1939, 1940).

GENERAL FEATURES OF THE VEGETATIVE SHOOT.—

The vegetative shoots of *Cunninghamia lanceolata* (Lamb.) Hook. consist of sturdy axes upon which the leaves are borne in a close spiral (fig. 1). The leaves remain on the stems for many years and at maturity are exceedingly leathery and sharp-pointed. They are reported to vary from 3 to 7 cm. in length (Pilger, 1926), but in the material available to the present writer none longer than 6 cm. was found. With the exception of *Sciadopitys* no other genus of the Taxodiaceae has leaves as long. There are lateral and terminal buds which remain dormant during the colder months; however, bud scales, although associated with strobili, do not occur on the vegetative shoots. All the leaves are of the same general form, viz., flattened linear-lanceolate, with minutely serrate margins, but those forming the outer constituents of resting buds are very short and scale-like. The limits of each recent yearly growth increment of the shoot can be determined by noting the position of these shorter leaves on the shoot axis (fig. 1).

STRUCTURE OF THE SHOOT APEX.—Strasburger (1872) referred to the shoot apex of *Cunninghamia* in connection with his work on *Araucaria brasiliana*. After describing a distinct protoderm, periblem, and plerome for the shoot apex of *Araucaria*, Strasburger wrote (1872, p. 325): "Annähernd ebenso wie *Araucaria brasiliana* verhält sich auch eine andere untersuchte Art, die *Araucaria Cunninghami* und in weiterer Folge auch *Dammara* und *Cunninghamia*." It is clear from Strasburger's discussion that he believed the apical meristem of *Cunninghamia* to be highly differentiated and stratified as in *Araucaria*. Perhaps it should be emphasized that the results of the present study are not in agreement with Strasburger's account. On the contrary, as is shown later, the shoot apex of *Cunninghamia* is the least stratified of any of the investigated genera of the Taxodiaceae, and possibly it may prove to be the primitive "type" in the family. This preliminary statement can be confirmed quickly by a casual observation of figures 2b to 10c, in which a zonation reminiscent of that described for seedlings of *Cycas revoluta* (Foster, 1939), is indicated.

In contrast to the hemispherical shoot apices of *Taxodium* (Cross, 1939) and *Cryptomeria* (Cross, 1941) the apical meristem of *Cunninghamia* is somewhat conical, and appears parabolic in longisection (fig. 3, 4, 8, 9, 10a, 10b). The apex is covered with a

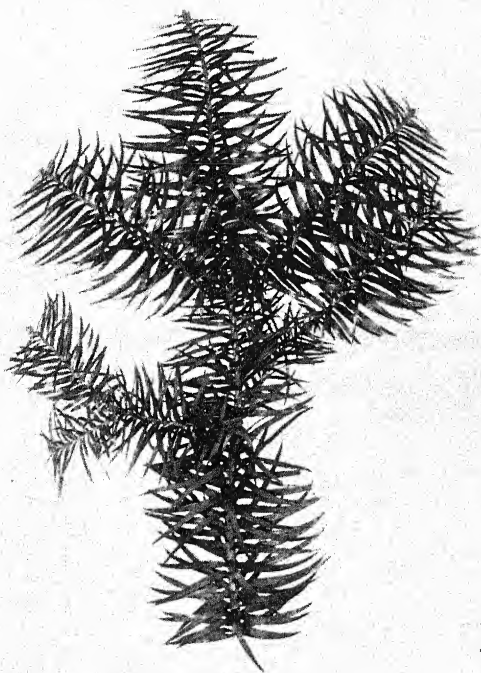


Fig. 1. Vegetative branchlets of *Cunninghamia*. $\times \frac{1}{2}$.

that have produced the highly specialized shoot apices found in modern angiosperms. Materials of all of the genera of the Taxodiaceae except *Glyptostrobus* have been assembled, and it is hoped that shoots of that genus may be obtained at an early date.

MATERIALS AND METHODS.—Materials were collected at weekly intervals during April and May of 1941 from young trees growing in a nursery² six miles south of Norman, Oklahoma. All materials were fixed under reduced pressure in a mixture of 5

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well-developed cuticle, detached remnants of which may be seen to the left of the tip in figure 10a. There is some seasonal variation in form, although this feature is not as well marked as it appears to be in some of the *Abietaceae* (Korody, 1937). In the winter condition the height (vertical distance of tip above axil of youngest leaf) averages about $50\ \mu$ (fig. 3). When active growth is resumed in the spring the height increases rapidly to about $80\ \mu$ (fig. 4, 10a). The average diameter of 45 apices (measured in a plane equidistant from the summit of the axis and the axil of the youngest foliar primordium) is $180\ \mu$. From this it can be seen that the shoot apex of *Cunninghamia* is

divisions remain as apical initials, and the inner derivatives augment the subapical portion of the meristem. The apical initials are often conspicuously larger than the cells of the subapical portion (fig. 10a, 10b), and the cellular patterns represented in figures 8 and 9 provide some evidence that anticlinal and periclinal divisions occur with approximately equal frequency. This is in contrast with the situation reported for the apices of the deciduous shoots of *Taxodium* and the shoots of *Cryptomeria*, for in these genera periclinal divisions in the surface layer of the apical meristem occur only infrequently, and are restricted to the extreme summit of the meristem.

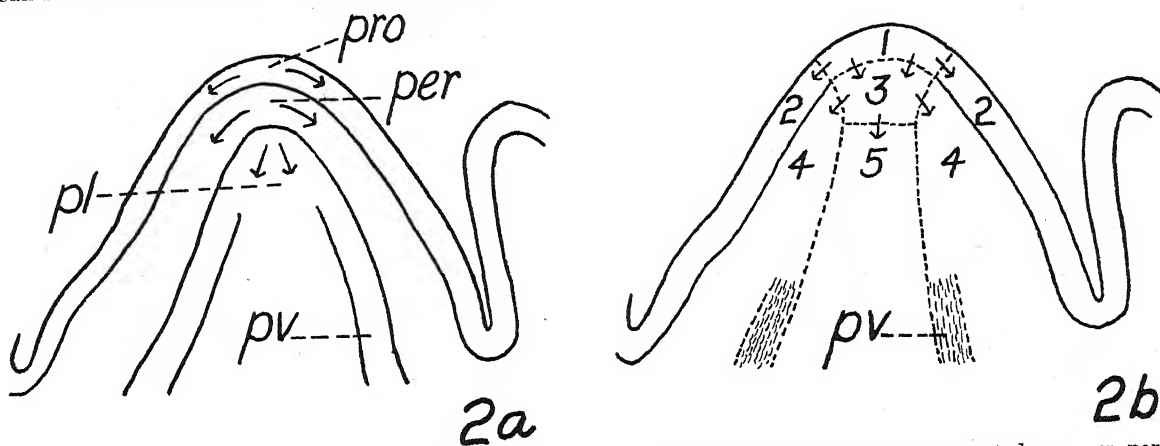


Fig. 2a-2b.—Fig. 2a. Diagram of longisection of shoot apex as described by Strasburger; *pro*, protoderm; *per*, periblem; *pl*, plerome; *pv*, provacular tissue.—Fig. 2b. Diagram of longisection of shoot apex as described in present paper, showing five growth zones; *pv*, provacular tissue.

generally larger than that of *Cryptomeria* (average width $90\ \mu$) but somewhat smaller than that of *Taxodium* (140 – $170\ \mu$ in width).

The surface layer of the apical meristem.—The surface layer of the shoot apex may be divided into two regions or growth zones (fig. 2b, zones 1 and 2). Zone 1, which is located at the summit and upper shoulders of the meristem, consists of a single tier of self-perpetuating apical initials which divide both periclinally and anticlinally (fig. 8, 9, 10a, 10b). All of the cells of the shoot are derived ultimately from this tier of apical initials. Zone 2, located on the lower shoulders and flanks, consists of a layer of cells in which periclinal divisions do not occur. The cells of this layer produce only the epidermal portions of the shoot, and, therefore, they may be regarded as a protoderm. These structural features are well illustrated by figure 3, which represents a nearly median longisection of a shoot apex in the winter condition. Even in the absence of mitotic figures, it is evident in the illustration that periclinal divisions have occurred abundantly in the surface cells at the summit (zone 1) but not in those of the lower shoulders and flanks (zone 2). Similarly, in figures 4, 5, 7, 8, 9, and 10b, prepared from expanding shoots, groups of cells that have divided periclinally may be distinguished at the summit and upper shoulders of the meristem. The outer derivatives resulting from such periclinal

Figures 6a, 6b, and 6c, drawn from transections of a shoot apex cut respectively at 8, 16, and $24\ \mu$ below the tip, will be of aid in understanding the structure of the apical initials and their inner derivatives. Figure 6a represents two apical initials and all portions of the surrounding cells that were visible in the section. Figure 6b represents the proximal portions of the two apical initials shown in figure 6a, a third apical initial, and several genetically related groups produced by anticlinal divisions in other apical initials. Figure 6c represents several genetically related groups (delimited by heavy lines) which have been formed by the inner derivatives of apical initials.

From a comparative point of view it should be emphasized that the apical initials of *Cunninghamia*, although lesser in number, resemble closely those of seedlings of *Cycas* (Foster, 1939). They are similar also to the apical initials of some genera of the *Abietaceae* (Korody, 1937). However, the presence of a protoderm on the lower shoulders and flanks of the shoot apex of *Cunninghamia* is a feature which contrasts sharply with these other genera.

The subapical portions of the shoot apex.—The subapical region consists of three growth zones (fig. 2b, zones 3, 4, and 5) which are distinguished by differences in position, plane of cellular division, and extent of vacuolation. Zone 3, derived directly from the apical initials of zone 1, consists of a group of

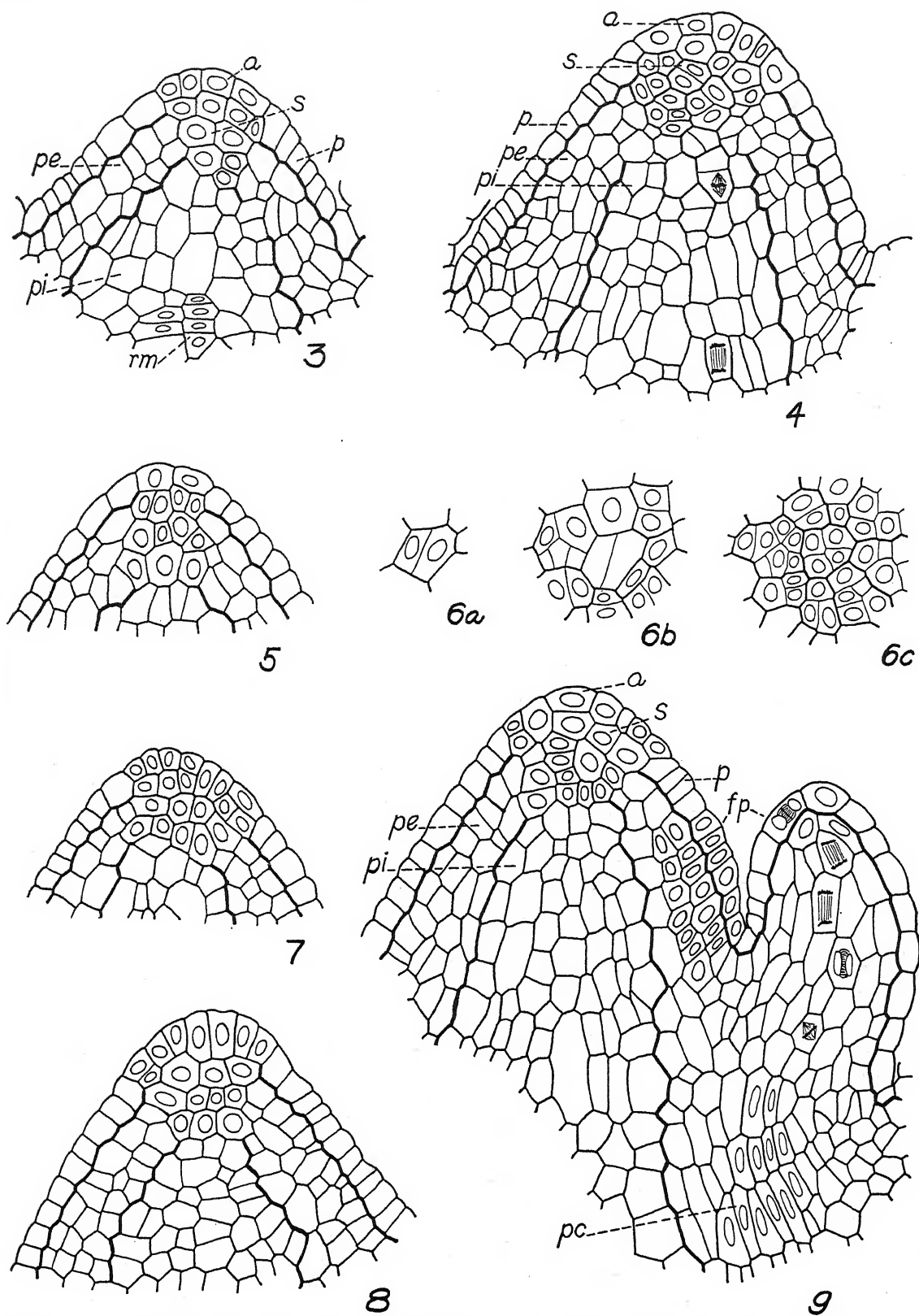


Fig. 3-9.—Fig. 3. Median longitudinal section of shoot apex of resting bud; *a*, apical initials; *s*, subapical mother cells; *p*, protoderm; *pe*, peripheral meristematic tissue; *pi*, pith mother cells; *rm*, rib meristem.—Fig. 4. Median longitudinal section of

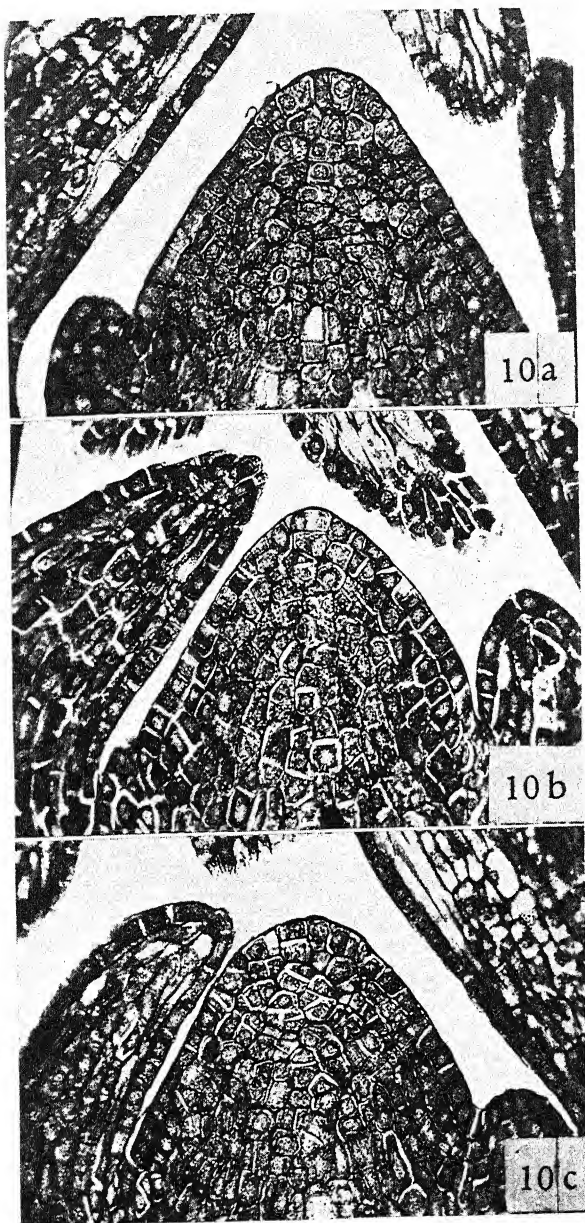


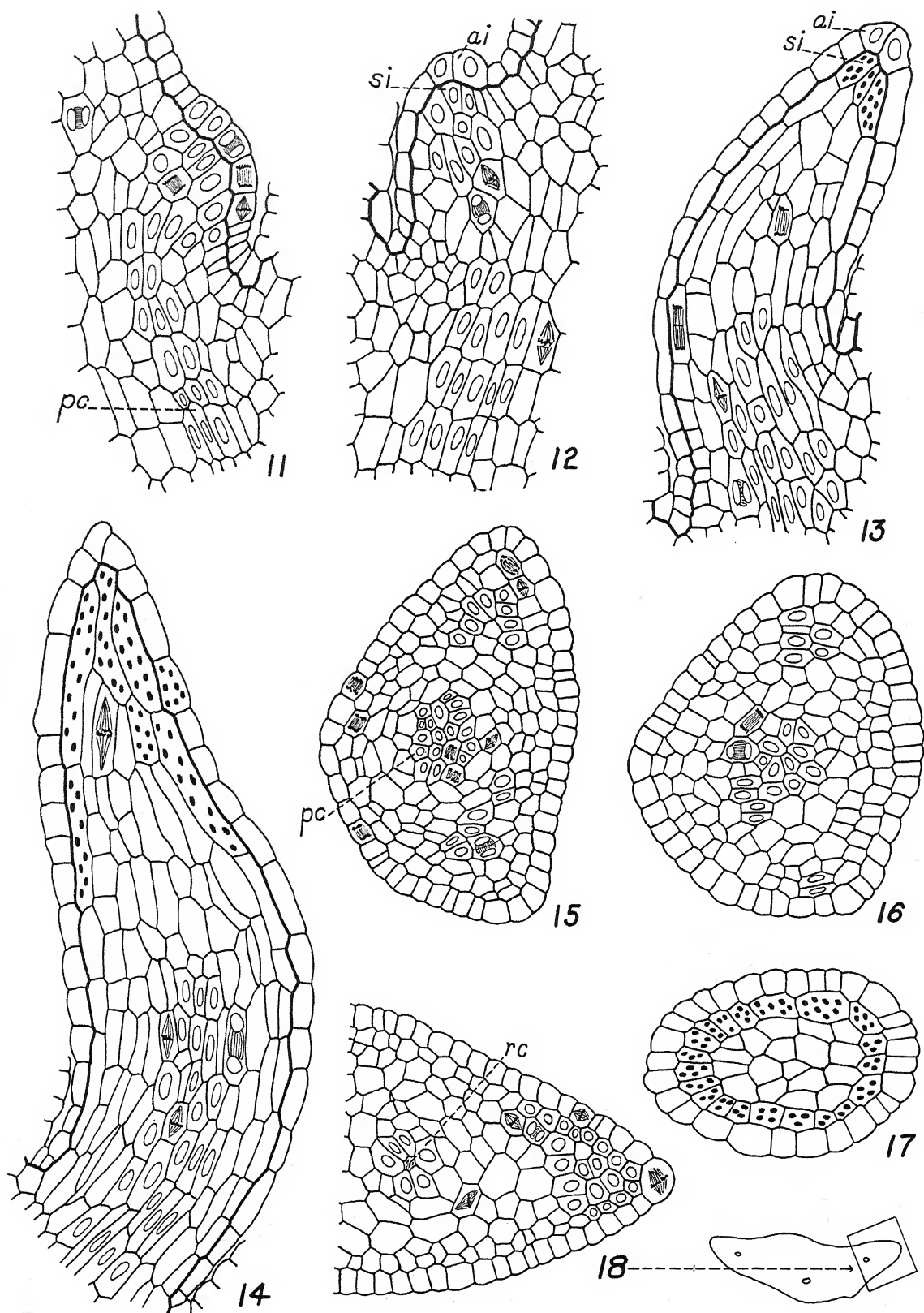
Fig. 10a–10c.—Fig. 10a. Median longitudinal section of apex of expanding shoot showing conspicuously enlarged apical initials, subapical mother cells, flanking protoderm, peripheral meristem, and pith mother cells.—Fig. 10b. Median longitudinal section of apex of expanding shoot showing periclinal divisions of apical initials.—Fig. 10c. Longisection of apex

apex of expanding shoot; note subapical groups of genetically related cells.—Fig. 5. Nearly median longitudinal section of apex of expanding shoot; note apparent sequence of divisions in apical initials.—Fig. 6a. Transection about 8μ below apex of expanding shoot showing two apical initials and portions of adjacent initials.—Fig. 6b. Transection 16μ below apex of expanding shoot showing proximal portions of the apical initials shown in figure 6a and groups of genetically related cells produced by vertical divisions in other apical initials.—Fig. 6c. Transection 24μ below apex of expanding shoot showing groups of genetically related cells produced from inner derivatives of apical initials.—Fig. 7. Nearly median longitudinal section of apex of expanding shoot showing evidence of a periclinal division in a surface cell on shoulder of apical meristem.—Fig. 8. Nearly median longitudinal section of apex of expanding shoot showing conspicuously enlarged apical initials.—Fig. 9. Median longitudinal section of apex of expanding shoot and young foliar primordium; *fp*, foliar primordium; *pc*, provascular tissue. All figures $\times 330$.

subapical mother cells (fig. 3, 4, 9, 10a, 10b, 10c) which are equivalent in position and function to the "subterminal mother cells" of *Cycas* (Foster, 1939) and to the central embryonic cells of *Taxodium* (Cross, 1939) and *Cryptomeria* (Cross, 1941). Lateral and basal derivatives of the subapical mother cells contribute to zones 4 and 5, respectively (fig. 2b, 3, 4, 8, 9), and these zones in turn produce all of the internal portions of the shoot. The subapical mother cells, except for their occasional smaller size (fig. 8, 9, 10a), are not easily distinguished cytologically from the apical initials. Although the cellular patterns in most of the apices examined gave positive evidence of recent divisions, mitotic figures were not often found, regardless of the time of day at which collections were made. However, because mitotic figures were abundant in young leaves (fig. 9, 11, 12, 13), it has been concluded that the shoots were in an actively growing condition. Possibly the puzzling absence of dividing cells in zone 3 (and other zones as well) may be correlated with periodicity of growth. Unfortunately, by the time the writer had found time to section the material, the growing season had ended, and additional collections could not be made to settle this point.

Zone 3 varies considerably with respect to the arrangement of its cells, but figures 4 and 9 represent conditions most commonly observed in the forty-five apices that were studied. In figure 4 the subapical zone is seen to consist of several groups of genetically related cells, delimited by heavy lines. Each of these groups of genetically related cells has been formed from an inner derivative of one of the apical initials. This was shown clearly in the stained sections, for the outline of the mother cell of each group could be distinguished easily because of its greater thickness. In figure 9 the arrangement of the cells in the subapical zone is somewhat different. Here some of the derivatives of the apical initials have enlarged, and one apparently has experienced a series of transverse divisions which has produced a vertical chain of four cells through the center of the zone. However, such examples of polarized growth are not of frequent occurrence, and usually the subapical mother cells divide irregularly in vertical, horizontal, and oblique planes (fig. 10c). The cellular pattern is often suggestive of a block meristem (Schüpp, 1926), and it conforms generally to the description given by Foster (1939) for the "subterminal mother cells" of *Cycas*.

of expanding shoot about 6μ from median, showing plasmolyzed subapical mother cells.



Zone 4 is a peripheral ring or cylinder of meristematic tissue, consisting of from two to four peripheral layers of densely staining cells, and located beneath the shoulders and flanks of the shoot apex. It is derived directly from the subapical mother cells (zone 3) and its derivatives produce all of the shoot except the pith and the epidermal portions. Because zones 3 and 4 merge into one another a sharp line of demarcation may not be drawn between them. However, in contrast to zone 3 anticlinal cellular divisions predominate in zone 4, and the cellular pattern as seen in longisection often appears somewhat stratified (fig. 4, 10b, 10c). However, this characteristic is not so well marked in *Cunninghamia* as it is in the meristematic flanks of *Taxodium* and *Cryptomeria*, and zone 4 is often very suggestive of the unstratified peripheral tissue of *Cycas* (compare figures 10a and 10b with Foster's figures 2 and 5, 1939).

Zone 5 consists of a core of pith mother cells which diverge from the basal portion of zone 3. The cells of this zone are arranged in irregular vertical rows. They are somewhat larger and more highly vacuolated than are those of zone 4, and they do not stain as densely. They enlarge very gradually as their distance from the summit is increased (fig. 9, 10a, 10b), in contrast to the pith mother cells of *Taxodium* and *Cryptomeria* (Cross, 1939, 1941) which often enlarge abruptly within three or four layers beneath the summit of the apical meristem. Except in the case of certain dormant apices (fig. 3), vertical files of cells suggestive of a rib meristem (Schüepp, 1926) were not observed. On the contrary the pith mother cells divide by the insertion of vertical as well as horizontal walls, and the lateral limits of the zone are, therefore, poorly defined (fig. 10a). The heavy lines which delimit zones 4 and 5 in the illustrations are drawn arbitrarily for the reader's convenience. The highly variable cellular pattern of the pith initials, together with their very gradual basipetal differentiation, suggest Foster's description of the central tissue in seedlings of *Cycas*.

It would seem that Strasburger's brief comment concerning apical meristem in *Cunninghamia* must have been based upon the study of poorly fixed material or upon non-median sections. The work of that period was done necessarily without the aid of modern technique, and in addition, botanical thought, insofar as it pertained to shoot apices, was dominated by the concepts of Hanstein (1868). A reinvestigation of much of the earlier work might prove profitable.

ONTOGENY OF THE FOLIAGE LEAF.—In general the origin and development of the foliage leaves of *Cunninghamia* are much like the corresponding processes

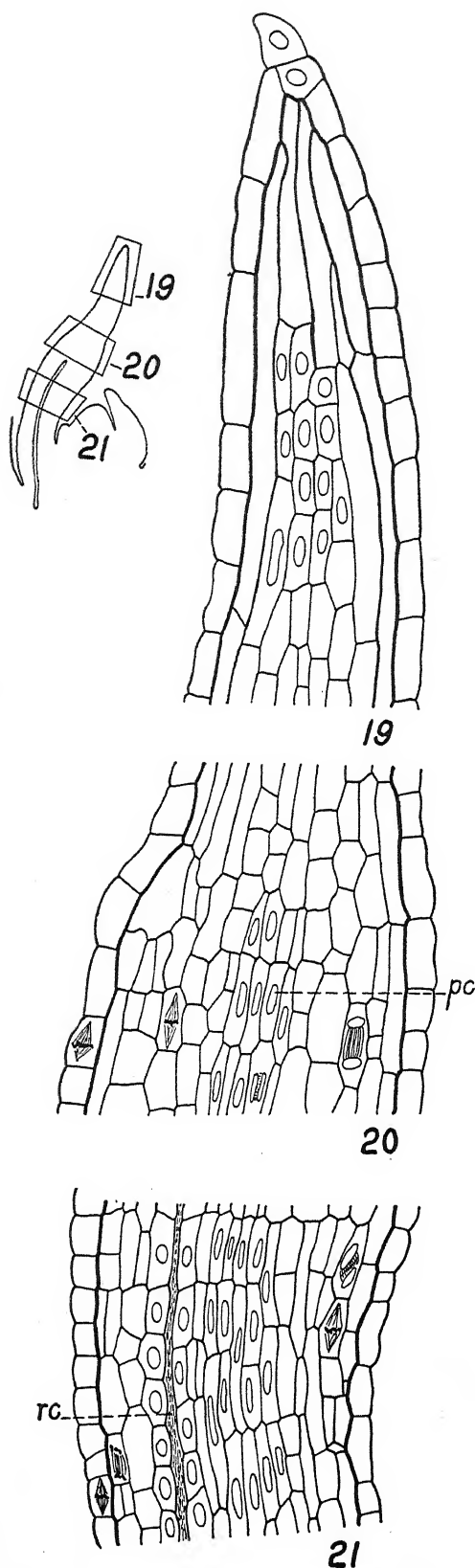
in *Taxodium* and *Cryptomeria*. However, there are enough differences to justify a reasonably comprehensive treatment. The following description pertains only to the large foliage leaves. The small, scale-like leaves that constitute the outer components of vegetative buds and the bud scales that enclose the strobili were not studied.

Initiation and early vertical growth of the primordium.—Foliage leaves are initiated in the flanks of the apical meristem from 80 to 100 μ below the summit. As in *Taxodium* and *Cryptomeria* the surface layer or protoderm does not divide periclinally during foliar initiation and forms, therefore, only the epidermal portions of the leaf. This contrasts significantly with the situation reported for other perhaps more primitive vascular plants, such as *Abies*, *Picea*, and *Pinus* (Korody, 1937), various other gymnosperms (Strasburger, 1872), and *Lycopodium* (Hartel, 1938), all of which have shoot apices that resemble those of *Cunninghamia* in that they do not have a discrete surface layer (protoderm or dermatogen) at the summits of their shoot apices. In *Abies*, *Picea*, *Pinus*, *Lycopodium*, and the genera of the Abietaceae investigated by Strasburger, periclinal divisions occur abundantly in the epidermal layer during foliar initiation, and the epidermal layer thereby contributes substantially to the internal portions of the primordium.

In *Cunninghamia* the initial elevation of a foliar primordium results from the lateral elongation of a number of cells located in the second and third layers of the primordium (fig. 11). This elongation usually is accompanied by considerable meristematic activity in the surface layer (fig. 11) and in the deep-lying cells (fig. 12), so that at least four layers of cells may be regarded as participating in the process of foliar initiation. At a very early stage in development the young leaf curves upward and begins to grow vertically. Figure 12 represents a young leaf just beginning upward curvature and vertical growth. In this figure two cells, conspicuous for their larger size, may be seen at the apex of the primordium. These are the apical initials, and they resemble closely the apical initials of the foliar primordia described for *Taxodium* (Cross, 1940) and *Cryptomeria* (Cross, 1941). Immediately beneath the apical initials in figure 12 are cells which correspond in position to the subapical initials in the young leaves of *Taxodium* and *Cryptomeria*. However, as is shown later, the subapical cells of the leaf of *Cunninghamia* are much less active mitotically than are those of the related genera.

A consideration of figures 10, 13, and 14, in the order given, should be of aid in understanding the

Fig. 11–18.—Fig. 11. Portion of median longisection of shoot apex showing initiation of leaf; *pc*, provascular tissue.—Fig. 12. Median longisection of young foliar primordium with adjacent portions of shoot axis; *ai*, apical initial; *si*, subapical initial of leaf.—Fig. 13. Median longisection of young leaf 160 μ high.—Fig. 14. Median longisection of young leaf 310 μ high.—Fig. 15. Transection 218 μ below tip of young leaf 250 μ high; note mitotic activity in submarginal zone and in the provascular tissue.—Fig. 16. Transection 154 μ below tip of young leaf 250 μ high.—Fig. 17. Transection 126 μ below tip of young leaf 250 μ high.—Fig. 18. Transection from proximal portion of young leaf 2,000 μ high; note metaphase in marginal initial and mitotic submarginal cells indicated by nuclei in drawing; *rc*, lateral resin canal. All figures $\times 330$.



vertical growth of the young leaf. In figure 10, which was drawn from a primordium about $75\ \mu$ high (measuring from the tip to the axil of the primordium), one of the apical initials is dividing anticlinally. The "subapical initials" are inactive mitotically, but lower in the primordium several cells in various stages of mitosis may be seen. Study of a great many leaves in similar stages of development has convinced the writer that early vertical growth of the foliar primordium is due to general mitotic activity throughout the organ, and is not a result of the mitoses of localized groups of cells, such as subapical initials. As a matter of fact, a subapical cell of the leaf of *Cunninghamia* was seen in a mitotic condition only once during the entire investigation. In figure 13, drawn from a median section of a foliar primordium about $160\ \mu$ in height, the subapical cells are represented in an early stage of differentiation as evidenced by their possession of densely-staining clumps of ergastic material. Lower in the same figure mitotic figures may be seen. The subepidermal cells of the adaxial and abaxial surfaces have begun to elongate in the distal portions of the primordium, and apparently these elongating cells do not experience further mitotic activity. In leaves of this height a tendency to restrict meristematic activity to the proximal half is quite noticeable. The tendency is expressed to a greater extent in figure 14, drawn from a primordium about $310\ \mu$ in height. Here the subapical cells are elongated conspicuously, and all of the cells of the distal half show evidence of elongation. However, these elongating cells are still potentially meristematic as is shown by the metaphase in the distal part of the leaf. Elongation in the subepidermal cells is especially marked and shows evidence of basipetal progression. From the smaller size of the cells, and the frequency of mitotic figures in the basal part of the leaf represented in figure 14, it is clear that meristematic activity is largely restricted to this region. After the $300\ \mu$ -stage, further increase in height, except that which is attained by continued cellular elongation, is largely a result of mitotic divisions in the lower one-half or one-third of the young leaf. However, the apical initials remain meristematic until the leaf is at least 1 mm. in height, and often one of them may divide at this late stage in such a plane as to produce a terminal, unicellular hair (fig. 19).

It is convenient to mention at this time that the provascular tissue of the young leaf differentiates acropetally. The provascular tissue of the subjacent internode is distinguishable at the time the foliar primordium is initiated (fig. 11). Probably it is continuous from the beginning with the provascular tissue of older portions of the shoot axis, but this could not be demonstrated with certainty. In any event, as far as the foliar primordia are concerned the provascular tissue differentiates in a strictly acropetal di-

Fig. 19-21.—Median longisections from various levels of foliar primordium $1,000\ \mu$ high; *pc*, provascular tissue; *rc*, median resin canal. $\times 330$.

rection (fig. 10–14). A similar situation has been reported by Crafts (1940) for the related genus *Sequoia*.

Lateral growth of the primordium.—During the earlier developmental stages the primordium is generally elliptical as seen in transections taken from various levels (fig. 15–17), although the adaxial surface may appear somewhat flattened in the proximal portions (fig. 15). Until a height of 200–250 μ is attained, only the median or axial portion of the mature leaf is represented. The origin of the lateral, photosynthetic portions occurs essentially as described for *Cryptomeria* (Cross, 1941). Some evidence of localized marginal and submarginal activity is supplied by figure 15, although a single row of submarginal initials such as occurs in the young leaves of *Taxodium* was not observed. Rather the young leaf appears to grow laterally as a result of the mitotic activity of submarginal strands of cells. Derivatives of the submarginal cells continue to divide and form genetically related groups of cells, which are often arranged in rows (fig. 15, 16) parallel with a line joining the vein and margin of the primordium. Lateral growth of this kind is restricted to the central and basal portions of the primordium (fig. 15, 16). It does not occur in the early maturing apex (fig. 17).

Although a plate meristem is not formed (Schüëpp, 1926), lateral extension through cellular divisions may occur until the leaf has attained a width of approximately 450 μ . The later phases of lateral extension, during which a final width of approximately 2.2 mm. is attained, are the result of cellular enlargement exclusively. The absence of a plate meristem in the foliar primordia of *Cunninghamia* may be correlated with the relatively narrow width of the mature leaf.

Many of the marginal initials enlarge conspicuously when the young leaf has attained a height of from 500 to 1,000 μ (fig. 18; Plate I, fig. 5). These enlarged initials, by anticlinal divisions, may contribute to the epidermal layer on either surface of the leaf (fig. 18). Ultimately, during maturation, many of them become mother cells of curious, marginal epidermal hairs (fig. 32, 33) which give the mature leaf a serrulate appearance.

Increase in radial thickness and differentiation of the axial portion.—The axial portion of the primordium does not increase markedly in radial thickness. Primordia 150 μ in height usually have a maximum radial thickness of 90 to 100 μ . As the height increases to 1,000 μ the radial thickness increases only to 140 or 150 μ . Therefore, a conspicuous midrib such as is characteristic of immature leaves of *Taxodium* and *Cryptomeria* does not develop. No indications of a localized adaxial meristem were observed; increase in radial thickness is due, first, to general meristematic activity throughout the axial portion and, finally, to cellular enlargement. The failure of an adaxial meristem has been noted also for *Taxodium* and *Cryptomeria*, and may be correlated with the fact that a petiole is not differentiated in the leaves of these genera.

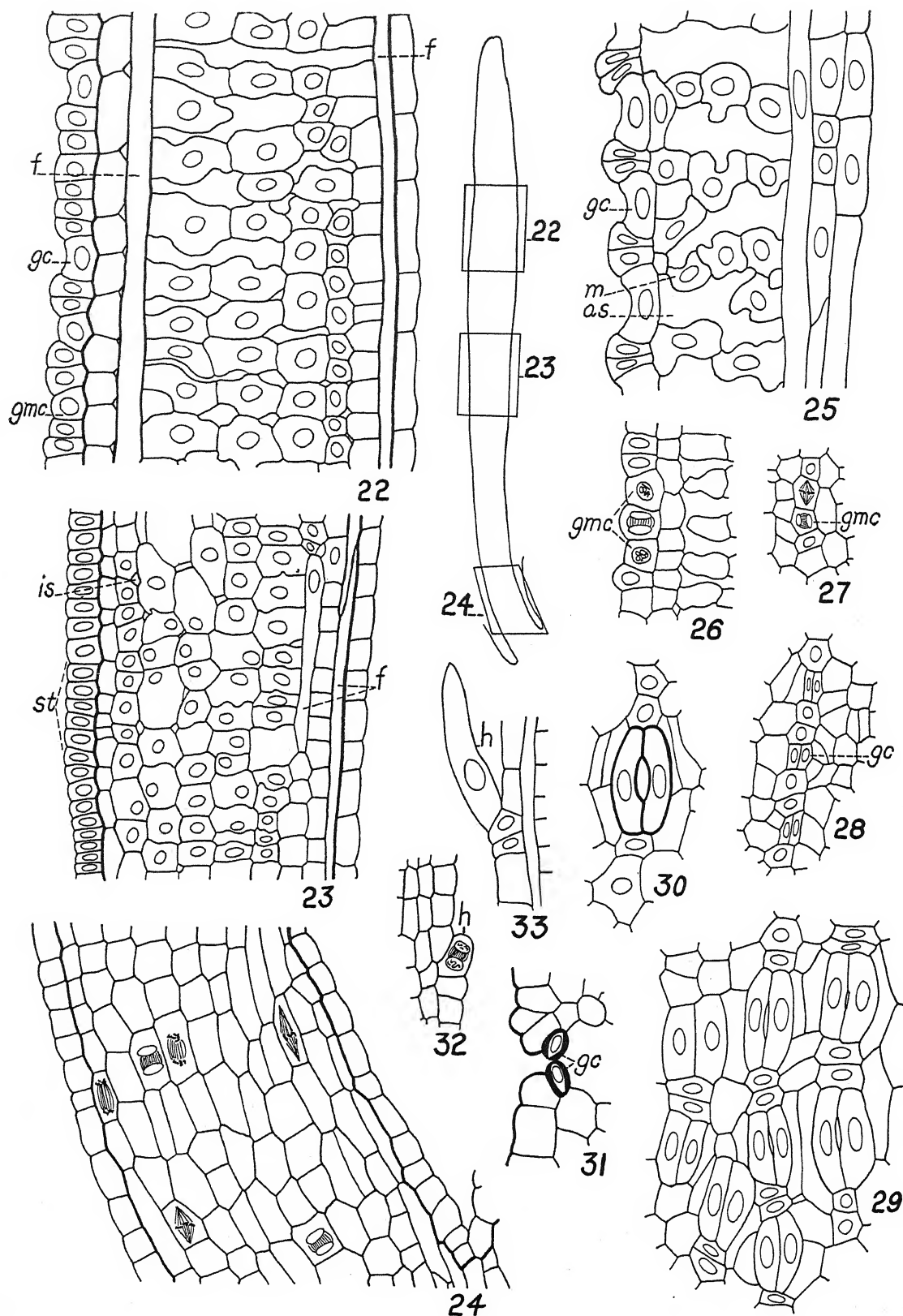
As mentioned earlier, the provascular tissue differentiates acropetally into the base of the primordium, where it may be distinguished easily when the organ is 150 μ in height. The median resin canal appears between the provascular tissue and the abaxial epidermis when the primordium is about 400 or 500 μ in height. The details of its origin and development are in general as described for *Taxodium* and *Cryptomeria*, but unlike these two latter genera the median resin canal of *Cunninghamia* does not end blindly in the cortex of the internode below but anastomoses with the resin canals of other leaves thus forming a continuous system.

Differentiation of the apex of the axial portion may be noticed as early as the 150 μ -stage (fig. 13) when the subapical cells stain densely because of their ergastic contents. Subsequently, the subapical cells begin to elongate (fig. 14). The subepidermal cells of both surfaces of the young leaf elongate similarly, beginning near the apex of the organ and proceeding basipetally. The elongating cells increase enormously in length, thrusting the apex of the leaf upward and forming a slender, tapering tip, the basal limit of which may be distinguished by its constricted appearance (fig. 14, 19, 20). The epidermal cells also elongate somewhat during the process.

Thus, the first cells that become mitotically inactive in the young leaf are the subapical cells. Starting at the subapex, differentiation in the form of cellular elongation proceeds basipetally in the subepidermal portions of the leaf axis until all of the subepidermal cells of the axial portion become greatly elongated. At maturity these subepidermal cells become fibers. The resin canal and provascular tissue do not differentiate in the constricted apical portion of the leaf.

Vertical growth and differentiation of the lateral or laminar portions.—The wing-like lateral portions are initiated when the primordium is from 200 to 250 μ in height, and for some time after initiation lateral and vertical growth occurs simultaneously as a result of cellular divisions. During this period of development, while the young leaf grows to a height of from 1,000 to 2,000 μ , cellular divisions occur in diverse planes throughout the lateral, laminar portions. However, growth in width by cellular division ceases early in ontogeny. Thereafter, the cells of the laminar portion (the axial portion as well) divide almost exclusively by the insertion of walls perpendicular to the longitudinal axis of the leaf. This results in the production of longitudinally oriented rows of cells (fig. 22–24) which have the appearance and structural features of a rib meristem (Schüëpp, 1926). The same condition obtains in *Cryptomeria* and to a very marked extent in *Taxodium*, where the tissues of the lamina are highly stratified because of the extraordinary lengths of the chains of cells.

Differentiation in the laminar portions is initiated in the distal regions near the tip of the leaf and proceeds basipetally. The main features of differentiation are described in the following paragraphs.



The epidermal cells of the adaxial surface cease dividing and elongate vertically until they are two or three times their original length. In the abaxial epidermis cellular elongation occurs also but is restricted to three vertical strips, one over the axial region or midvein and one near each margin of the young leaf. This leaves two strips of mitotically active epidermal cells, one on each side of the midvein and occupying much of the area between the midvein and leaf margin (Plate I, fig. 4). These actively dividing strips of cells ultimately produce rows of guard cell initials (fig. 22, 25, 27–29). The stomata of the abaxial surface are restricted to these areas. Stomata also appear in small isolated groups, near the midvein on the adaxial surface. Another feature of epidermal differentiation is the enlargement of the marginal initials, to which reference has been made earlier.

The subepidermal cells of the lamina elongate also and at maturity are many times their original length, except in the regions immediately beneath the stomatiferous strips where they elongate only slightly (fig. 22, 23).

Most of the internal cells of the laminar portion become chlorenchymatous; however, certain of them elongate and ultimately become fibers (fig. 22, 23, 25). Also a lateral resin canal is differentiated vertically in each half of the young leaf about 125 μ from the margin (fig. 18). These lateral resin canals differentiate basipetally as the leaf is elongated by the basal meristem. They also differentiate acropetally to a certain extent. The presence of three resin canals in each leaf of *Cunninghamia* is distinctive (Plate I, fig. 2) because in *Taxodium* and *Cryptomeria* there is only one. The photosynthetic cells enlarge greatly, but do not keep pace with the vertical growth of the epidermal and subepidermal layers. Therefore, they are placed under stress and soon are stretched until air spaces are formed at their corners (fig. 23). Ultimately the photosynthetic cells separate into transverse anastomosing plates of much the same type as described for *Taxodium* and *Cryptomeria* (fig. 22, 25).

It has been emphasized that differentiation in the laminar portions, starting near the tips of young leaves, proceeds in a basipetal direction. The basal portion of the leaf is meristematic and remains so until the organ is completely mature. Figures 22 to 24 illustrate differentiation in the distal, middle, and

proximal portions of a lamina $3\frac{1}{2}$ mm. in height, as seen in longitudinal section.

Maturation.—The apex of the leaf matures first. The walls of the epidermal cells thicken and become cutinized. The elongated subapical cells are stiffened by the thickening of cell walls (fig. 19), and when the leaf has attained a height of 1 mm. or even less, the tip is already stiff and sharp.

The apical initials are the last cells of the apex to mature (fig. 19). Ultimately one of them usually divides by the insertion of an oblique wall, and the outer derivative elongates forming a stiff hair filled with densely-staining spherical bodies. Similar stiff hairs are formed from many of the marginal initials, as is mentioned earlier. Figure 32, drawn from a section cut parallel with the surface of a leaf, represents a protruding, marginal initial which is undergoing an oblique division. The outer derivative forms an elongated, pointed, stiff hair, and the inner derivative becomes a basal cell as is represented in figure 33. The hairs are formed successively in a basipetal direction. They appear as minute serrations on the margins of the mature leaf.

The stomatiferous strips mature in an interesting manner. In the younger portions of a leaf the cells in these areas are similar in size and structural features (fig. 23, 27), but in the older distal regions there are rows of guard cells separated by epidermal cells which are considerably altered in size and shape (fig. 29). For a study of stomatal development it proved advantageous to use sections cut parallel with the surface of a leaf. The stomata are arranged in parallel rows, and it was easy to follow a row from a region of greater maturity to one of relative immaturity. Figures 27 to 30 illustrate various stages in the development of stomata. The guard cells are oriented with their long axes parallel with the longitudinal axis of the leaf. Each pair of guard cells is usually separated from its neighboring guard cells in the same row by two other epidermal cells. Mitoses which produce this situation are represented in figures 26 and 27. A later stage, with guard cells formed but not enlarged, is represented in figure 28. Figure 29 represents a portion of the epidermis with various ages of guard cells, some of them beginning to separate. A mature stoma is portrayed in figure 30. As the epidermis matures, the cells which separate the stomata in the various rows protrude above the surface of the leaf, apparently squeezed out of position by the elongation of the guard cells (fig. 25, 26). At

Fig. 22–33.—Fig. 22. Longisection of portion of lamina from distal half of leaf 2,000 μ high; *f*, fiber; *gc*, guard cell; *gmc*, guard cell mother cell. Note conspicuous air spaces between cells of mesophyll.—Fig. 23. Longisection of portion of lamina about midway between tip and base of leaf represented by figure 22; *is*, intercellular space; *st*, stomatiferous cells; *f*, fiber.—Fig. 24. Longisection from lower portion of lamina of leaf represented in figure 22; note organization of rib meristem.—Fig. 25. Longisection near margin of lamina of nearly mature leaf; *m*, cell of mesophyll; *gc*, guard cell; *as*, air space.—Fig. 26. Longisection perpendicular to surface through stomatiferous area; *gmc*, guard cell mother cell.—Fig. 27. Portion of stomatiferous layer cut parallel with surface showing origin of guard cells and intervening cells.—Fig. 28. Section of stomatiferous layer cut parallel with surface showing recently formed guard cells.—Fig. 29. Section of stomatiferous layer cut parallel with surface showing maturing guard cells.—Fig. 30. Surface view of stoma and guard cells in nearly mature condition.—Fig. 31. Transection of guard cells and adjacent epidermal cells.—Fig. 32. Portion of margin of young leaf from section cut parallel with the surface showing origin of epidermal hair from marginal initial; *h*, hair.—Fig. 33. Section of margin of leaf cut parallel with the surface showing young hair and basal, sister cell. All figures $\times 330$.

maturity the guard cells are sunken and covered partially by surrounding epidermal cells (fig. 31).

The subepidermal cells, with the exception of those beneath the stomatal areas, develop into enormously elongated, thick-walled fibers. Some of these fibers from macerated material exceeded 3 mm. in length.

The maturation of the mesophyll is essentially as described for *Taxodium* and need not be redescribed here. The elongated cells scattered in the mesophyll, often occurring in groups of from two to seven, mature into thick-walled fibers. The abundance of mechanical tissue found in the leaf of *Cunninghamia* results in a very tough and stiff photosynthetic organ, much in contrast to the soft, flexible leaves of *Taxodium* where mechanical tissue is lacking or poorly developed.

The lateral resin canals mature simultaneously with the chlorenchyma. In structure they are similar to the median one, though somewhat smaller (Plate I, fig. 2). As the basal portion of the leaf loses its meristematic character, the lateral resin canals differentiate basipetally through this region, and, when the leaf is completely mature, they may be observed in the cortex of the shoot axis. However, in contrast with the median canal, they end blindly and do not anastomose with other canals. At least no evidence of anastomosing was observed during the present study.

GENERAL ANATOMY OF THE MATURE FOLIAGE LEAF.—Bertrand (1874) published an illustration and a brief description of the anatomy of the mature leaf of *Cunninghamia*. Later Mahlert (1885) and, more recently, Feustel (1921) have contributed very brief accounts without illustrations.

In transection the distal half of the mature leaf resembles an elongated, distorted ellipse (Plate I, fig. 1). The proximal half is often compressed radially, and appears somewhat scale-like (Plate I, fig. 2). The walls of the epidermal cells, especially the outer surfaces, are greatly thickened with a material which stains red with safranin. Pits are very conspicuous especially in the epidermal cells near the margins of the leaf. The stomata are arranged in parallel rows on the abaxial surface, and occur infrequently in scattered areas on the adaxial surface. The guard cells are parallel with the longitudinal axis of the leaf. They are sunken and have heavily-thickened walls (Plate I, fig. 3).

There is a conspicuous hypodermal sheath consisting of cells with walls so thickened that the lumina are often represented by slits or points (Plate I, fig. 6). A similar hypodermal layer has been reported for *Cryptomeria* (Cross, 1941). The walls of the hypodermal cells do not stain with safranin but absorb fast green. The hypodermal layer generally consists of one layer of cells, but it is double in the margins and occasionally it may be double locally elsewhere. The thickened cells are not found in the vicinity of stomata.

The mesophyll consists of well-developed palisade and spongy tissue (Plate I, fig. 1, 5, 6), and the cells have thick walls with simple pits. The palisade con-

sists of two or more layers near the margins; consequently, the leaf is thicker in these regions (Plate I, fig. 1). Many of the cells of the spongy mesophyll are elongated transversely (laterally). Very long, thick-walled fibers are irregularly distributed throughout the mesophyll, occurring either singly or in groups of from two to seven. The fibers are structurally very similar to the fibers of the hypodermal layer. Lateral resin canals are found in the mesophyll of the proximal portions of the leaf, but these are not usually present in sections from the distal half (compare figures 1 and 2 in Plate I). In transection the palisade and spongy tissues are continuous past the vascular bundle as in *Cryptomeria*.

The vascular bundle is large and well developed (Plate I, fig. 6). The cells of the xylem occur in rows, and apparently all of the elements are secondary in origin. With respect to wall thickenings various types of spiral depositions were noticed in the first-formed elements, and some cells with thickenings partially spiral and partially annular were observed. The cells of the later-formed xylem have bordered pits on their radial walls. The xylem is limited toward the adaxial surface by a zone of very thin-walled cells. The cells of this zone are very delicate, and because of technical difficulties usually appeared somewhat crushed in the preparations.

The cells of the functional phloem, like the xylem, are also arranged in rows and are probably secondary in origin. The first-formed phloem, possibly primary phloem, may be located as a zone of crushed cells in Plate I, figure 6. Immediately adjacent to the phloem toward the abaxial surface is the large median resin canal consisting of a cylinder of secretory elements enclosed by a sheath of slightly thickened cells.

Strands of transfusion tissue with well-developed, bordered pits flank the xylem and phloem. The entire bundle is surrounded by a sheath of cells which resemble those of the mesophyll with respect to shape but which have conspicuously thickened walls. This "bundle sheath" varies in thickness from one to several layers (Plate I, fig. 3, 4).

DISCUSSION.—The surface layer at the summit and upper shoulders of the apical meristem of *Cunninghamia* consists of cells which divide both anticlinally and periclinally. However, on the lower shoulders and flanks a discrete surface layer or protoderm is differentiated. Thus, with respect to the surface layer, the shoot apex of *Cunninghamia* seems to represent a condition phylogenetically intermediate between the apices of the Abietaceae and those of *Taxodium* and *Cryptomeria*. The evidence indicates that an entirely discrete surface layer may be evolving in the apical meristem of the Taxodiaceae as a result of a gradual diminution and ultimate loss of periclinial divisions in the outer cells of the shoot apex. The loss of periclinial divisions apparently occurs first in the sides of the shoot apex (*Cunninghamia*), then proceeds acropetally into the flanks or shoulders (deciduous shoots of *Taxodium* and shoots of *Cryptomeria*), and finally reaches the cells of the summit

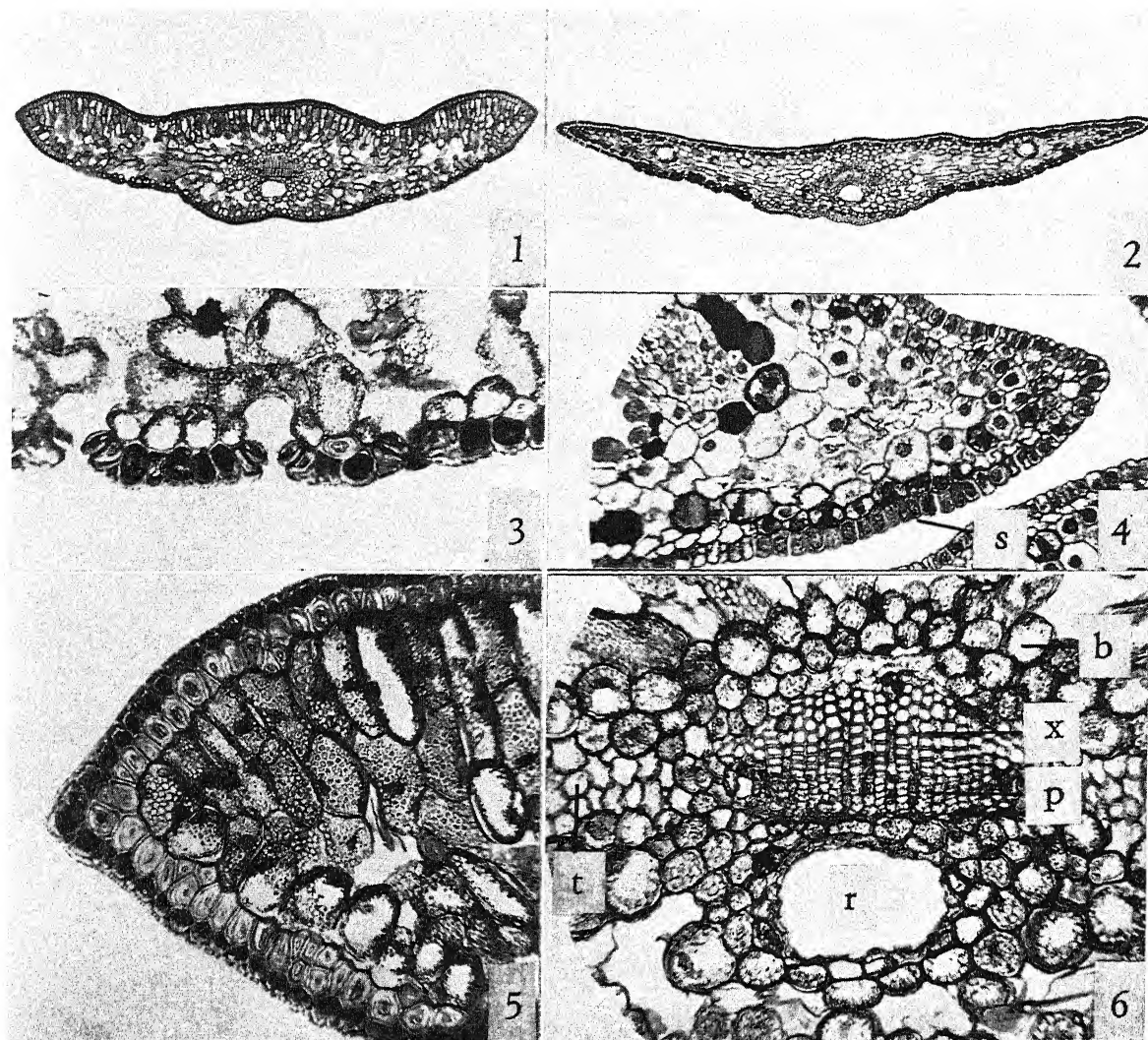


Plate I.—Fig. 1-6.—Fig. 1. Transection of distal half of mature foliage leaf; note absence of lateral resin canals. $\times 38$.—Fig. 2. Transection of proximal half of maturing foliage leaf; note presence of lateral resin canals and laterally elongated cells of mesophyll. $\times 38$.—Fig. 3. Transection of leaf showing stoma and guard cells; note thickened, pitted walls of cells of mesophyll. $\times 250$.—Fig. 4. Transection of young foliar primordium showing marginal initials and abaxial, stomatiferous layer; s, stomatiferous layer. $\times 250$.—Fig. 5. Transection of marginal portion of leaf showing details of epidermis, hypodermal layer, palisade, and spongy mesophyll. $\times 250$.—Fig. 6. Transection of mature vascular bundle and resin canal; t, transfusion tissue; b, bundle sheath; x, xylem; p, phloem; r, resin canal. $\times 250$.

(permanent shoots of *Taxodium*). The entirely discrete surface layer at the shoot apex of the permanent shoots of *Taxodium* may be regarded as a tunica (Cross, 1939), a protoderm (Strasburger, 1872) or a dermatogen. Perhaps it should be suggested at this time that the entire apex of the permanent shoot of *Taxodium* (Cross, 1939; Pl. 9, fig. 3), with its uniseriate tunica and clearly differentiated subterminal regions or zones, resembles definitely the shoot apex of *Opuntia cylindrica* (Boke, 1941; fig. 2). The differences appear to be quantitative rather than qualitative, and, if additional researches prove this to be the case, it should be possible to correlate at least some of the apparently divergent "types" of cellular

patterns found in the shoot apices of coniferous and angiospermous genera.

The subapical mother cells in the shoot apex of *Cunninghamia* are perhaps more clearly defined than in any other investigated coniferous genus. They are equivalent in origin, position, function, and some structural features to the "subterminal mother cells" in the shoot apices of seedlings of *Cycas revoluta* (Foster, 1939). As a matter of fact, the entire shoot apex of *Cunninghamia* is structurally and functionally suggestive of that described for the seedlings of *Cycas*, if one disregards differences in size and the presence of a protoderm on the flanks of the apex of *Cunninghamia*. Foster (1939) has pointed out that

with respect to shoot apices "probably the closest resemblance to *Cycas* is shown by certain of the Abietineae." However, using the shoot of *Abies venusta* as an example, Foster emphasizes that there are significant differences, particularly with respect to cellular size and arrangement. Specifically, the cells of the peripheral tissue in *Abies* are more stratified, and the peripheral tissue is, therefore, more rigidly delimited from the central tissue (pith mother cells) than in *Cycas*. Because of these differences Foster is inclined to question the equivalence of the various zones in the shoot apices of *Abies* and *Cycas*. If similar comparisons are made using *Cycas* and *Cunninghamia*, it is apparent at once that the points of contrast listed by Foster are much less marked. A comparison of Foster's figures of *Cycas* with those of *Cunninghamia* will show that differences in cellular size may be discounted, and that the two growth zones (peripheral and central tissues) are not always more clearly delimited in *Cunninghamia* than in *Cycas*.

I should like to suggest also that the use of structural features such as "cellular size," "degree of stratification," etc., in determining the equivalence of growth zones should be used with caution, because considerable variation with respect to these characteristics is generally known to exist in the apices of related genera, and even in apices of species belonging to the same genus. It seems likely that characteristics such as origin, position, and function may constitute reasonably reliable criteria for establishing the equivalence of growth zones in the shoot apices of the various genera of vascular plants, and from this point of view the similarities of *Cunninghamia* and *Cycas* appear to be noteworthy.

Thus, apparently there is some possibility that the shoot apices of the various genera of Taxodiaceae (particularly *Cunninghamia* and *Taxodium*) may serve to articulate the uniquely zoned apices of the cycads with those of certain angiosperms, and a tentative "series" (not phylogenetic) consisting of *Cycas*, *Cunninghamia*, *Taxodium*, and *Opuntia*, arranged in the order of their similarities, might provide a basis for additional inquiry. However, it must be emphasized that much work remains to be done be-

fore the relationships, if any, can be elucidated with confidence.

SUMMARY

The apical meristem and the ontogeny of the foliage leaf of *Cunninghamia* are described. Strasburger's (1872) concept of the shoot apex, i.e., that it consists of a protoderm, periblem, and plerome, is not supported, since no indication of these histogens was found in any of forty-five apices. On the contrary the apex appears to consist of five poorly delimited growth zones. There is a single tier of apical initials (zone 1) which occupies the summit and upper shoulders. Lateral derivatives of the apical initials produce a protoderm (zone 2) in which anticlinal divisions predominate. Basal derivatives of the apical initials become the subapical mother cells (zone 3). Derivatives of the subapical mother cells give rise to the peripheral, or flanking, meristem (zone 4) and to the pith mother cells (zone 5). The peripheral tissue produces the cortex and probably most of the vascular tissue. It is concluded that the shoot apex of *Cunninghamia* is less specialized and, therefore, probably more primitive than are those of *Taxodium* and *Cryptomeria*. It is similar structurally to the shoot apex described for seedlings of *Cycas revoluta* (Foster, 1939).

The origin and development of the foliage leaf are essentially as described for *Taxodium* and *Cryptomeria*. Neither an adaxial meristem nor a plate meristem is developed, a fact which may be correlated with the restricted radial and lateral dimensions of the mature leaf. A basal rib meristem is formed early in ontogeny, and the derivatives produce most of the internal tissue of the leaf. The resin canals of *Cunninghamia* present contrasting features in that each leaf has two lateral and one median canal, whereas leaves of *Taxodium* and *Cryptomeria* have a single canal each. Further, the median canal in the leaf of *Cunninghamia* anastomoses with canals from other leaves in the cortex of the shoot axis, although this is not true of the lateral ones.

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THE CHROMOSOMES AND NUCLEOLI OF MEDEOLA VIRGINIANA¹

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MANY OF the members of the genera *Trillium* and *Paris* have been the subject of much valuable cytological research. It seemed probable, therefore, that *Medeola*, a closely related genus, might also furnish favorable material for similar studies. This paper is a report of the observations of the chromosomes and nucleoli of *Medeola virginiana*, the only species of the genus. As far as the authors are aware no previous reports of the chromosomes of this species have been made.

The flower buds were collected late in June from about fifty scattered plants growing in the vicinity of College Park, Maryland, and this material was preserved in a 3:1 absolute alcohol-acetic acid mixture. Preparations were made from this preserved material and from fresh material by the use of McCallum's Iron-propionocarmine (Johansen, 1940).

When observations were made of the metaphase and anaphase stages of mitosis in the young ovary wall, fourteen chromosomes were present (fig. 1 and 2). Similar observations were made of metaphase I of meiosis (fig. 6) and the metaphase of the first microspore division (fig. 10). The presence of seven chromosomes in both of these stages confirmed the diploid counts. Some tapetal cells were located which had twenty-eight chromosomes and were, therefore, tetraploid.

Like the chromosomes of both *Trillium* and *Paris*, those of *Medeola* are large, and, since the kinetochores are plainly visible, it was possible to measure accurately the length of the arms as well as the length of the whole chromosomes. In this way each chromosome could be easily identified and a ratio established (Woods and Bamford, 1937) for it. The seven chromosomes, arranged in order of total length, were then arbitrarily designated (fig. 12) as follows:

Chromosome A, ratio 1.10	Chromosome D, ratio 3.06
Chromosome B, ratio 1.42	Chromosome E, ratio 9.11
Chromosome C, ratio 2.22	Chromosome F, ratio 1.14
Chromosome G, ratio 1.72 (secondary constriction, short arm)	

The most interesting part of these observations was the nucleolar-chromosome relationship which could be followed closely. In the early telophase of mitosis two small nucleoli were observed attached to the G chromosomes. Later in this same stage and during the following resting stages the nucleoli were present, but their chromosome attachments could not

be observed. Apparently some fusion takes place because certain cells contain one large nucleolus and others two smaller ones. In the very late prophase of mitosis one pair of chromosomes (G) could be seen attached to one (fig. 5) or two nucleoli at their secondary constriction. In the case of the tetraploid cell of the tapetum in a late prophase or early metaphase, four nucleoli could occasionally be observed and each was connected to one G chromosome (fig. 3). During the late diakinesis stage of meiosis the bivalent G chromosome was frequently observed with the one nucleolus attached to it (fig. 4). The interphase between the two meiotic divisions is quite marked and nucleoli are formed. During prophase II two nucleoli could be observed, and one was attached to each of the two chromatids (fig. 11) of chromosome G. At a later stage of this same division, late anaphase or early telophase, a similar observation could be made. Here a single nucleolus is attached to the G chromosome (fig. 7). During the prophase of the first microspore division a nucleolus was again observed (fig. 13) attached to chromosomes G. In all the above cases the nucleolus was located at the secondary constriction on the short arm (fig. 5) of this chromosome.

In the mid-prophase of the first division of meiosis we have been unable to recognize the individual chromosomes but in such preparations only, the "nucleolar-organizing body" is clearly visible (fig. 8 and 9), attached to a single nucleolus in each case.

The chromosome morphology of both *Trillium* and *Paris* has been described by Gotoh (1933), Haga (1934), Warmke (1937), Matsuura (1938), Darlington (1941), and many others. The five chromosomes in the haploid complement of the diploid species in these two genera appear identical except for the presence of a satellite or secondary constriction chromosome D in *Paris* (Haga, 1934; Matsuura, 1938; Darlington, 1941). Chromosomes A, B, C, and E of *Trillium* and *Paris* are apparently like chromosomes A, C, D, and F in the present species. Chromosome D of *Trillium* is not comparable to any chromosome in *Paris* but is like chromosome E in *Medeola*. Also chromosome D of *Paris* is not comparable to any chromosome in *Trillium* but is like chromosome G of *Medeola*. These are the nucleolar chromosomes in both *Paris* (Matsuura, 1938) and *Medeola*, and have a secondary constriction in the short arms. Chromosome B of *Medeola*, or a like chromosome, is absent in *Paris* and *Trillium*. The

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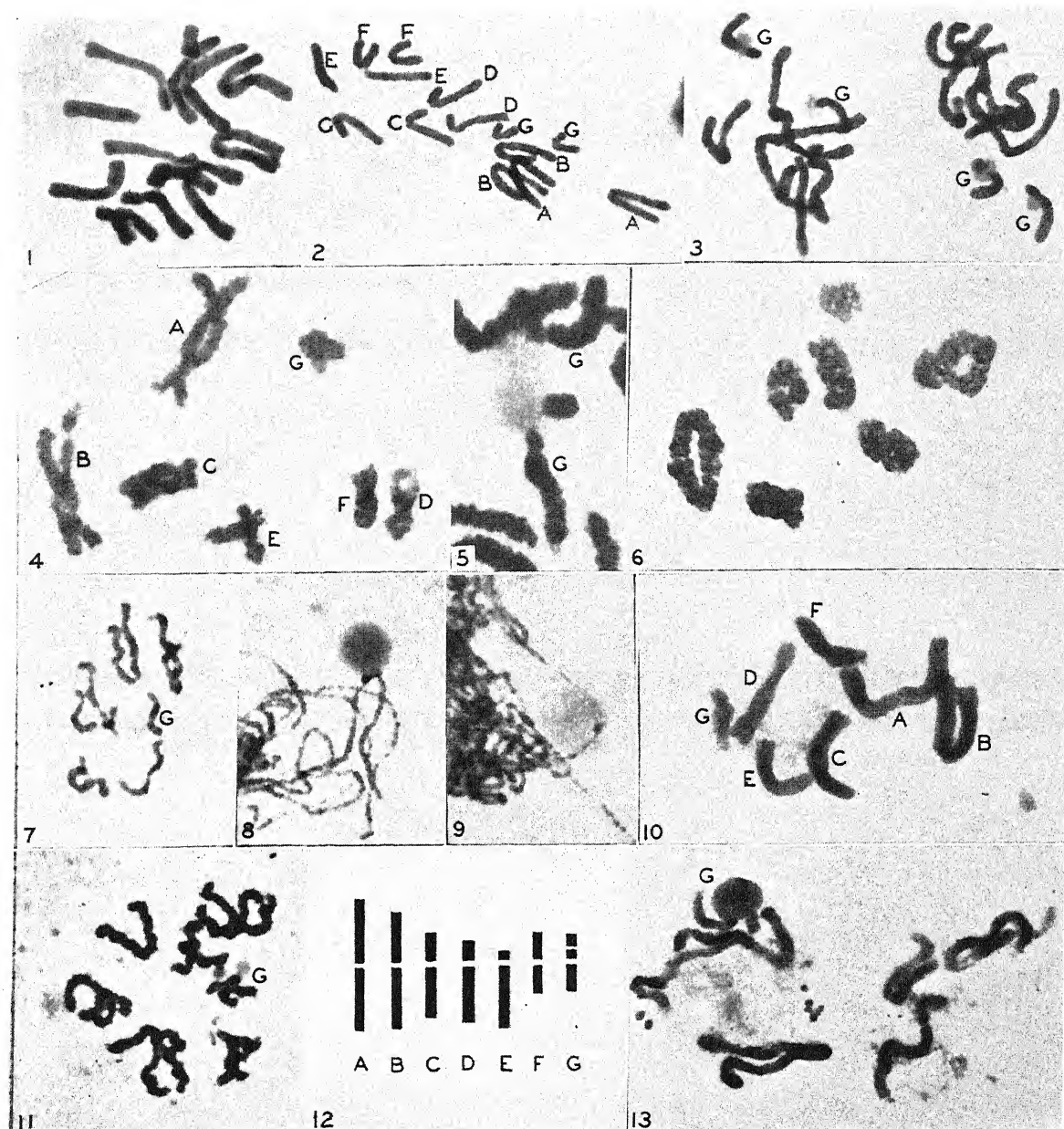


Fig. 1-13.—Fig. 1. Mitotic metaphase in young ovary wall. $\times 1,095$.—Fig. 2. One-half anaphase of mitosis in young ovary wall. $\times 1,095$.—Fig. 3. Part of a tetraploid tapetum cell in late prophase showing the four G chromosomes, each attached to a nucleolus. $\times 1,095$.—Fig. 4. Late diakinesis of metaphase I. The nucleolus is still present attached to the G bivalent. $\times 1,095$.—Fig. 5. Two mitotic prophase chromosomes (G) attached to a single nucleolus at their secondary constrictions. $\times 2,140$.—Fig. 6. Late metaphase of meiosis I. The seven bivalents are just beginning to separate. $\times 1,095$.—Fig. 7. Telophase of meiosis II. The nucleolus on chromosome G. $\times 1,095$.—Fig. 8 and 9. Mid-prophase I of meiosis. The deep-staining nucleolar-organizing body is visible on the surface of the nucleolus in each photograph. $\times 1,095$.—Fig. 10. Metaphase of the first microspore division. $\times 1,260$.—Fig. 11. Prophase II of meiosis. Nucleoli formed at interphase are still attached to each chromatid of chromosome G. $\times 1,095$.—Fig. 12. Idiogram from averages of somatic chromosomes. $\times 1,095$.—Fig. 13. Late prophase of first microspore division showing nucleolus attached to chromosome G. $\times 1,095$.

similarity in chromosome complements of these three genera is interesting in view of their generally accepted close relationship from a taxonomic standpoint, but at the same time the differences in both

morphology and number of the chromosomes indicate that they are separate and distinct forms.

The question of the chromosome-nucleolus relationship has been surveyed recently by many au-

thors, particularly by Gates (1937, 1939), his students (Nandi, 1937; Bhatia, 1938; Iyengar, 1939; Mensinkai, 1939; Jacob, 1940; Sikka, 1940; Pathak, 1940; Bhaduri, 1941), and many others (Fernandes, 1935; Woods, 1937; Resende, 1937, 1939; Sato, 1938, 1939; Matsuura, 1938). It seems unnecessary to discuss it in detail here again. The majority of these recent investigations have tended to strengthen the basic idea of Heitz (1931). As Warmke (1941) has recently pointed out, there is "a definite and constant relationship between secondary constrictions, including satellite constrictions, and the formation of nucleoli." Resende (1937) made an extensive survey of many species and varieties of the higher plants and concluded that there is a SAT-region—the satellite or secondary constriction thread—in at least one chromosome of the complement in all plants, and that the nucleolus is formed on that constriction thread. In some species of *Trillium* he was unable to find this but suggested that it was due to the extremely small size.

One of the most questionable phases of the nucleolus and chromosome association is the exact point of nucleolar organization and whether the constriction thread organizes the nucleolus or the organization of the nucleolus results in the constriction thread. Many workers, Heitz (1931) and Resende (1937) among them, have concluded from observation alone that the nucleolus is organized on or by the constriction thread. McClintock (1934), on the

basis of various experimental evidence, demonstrated clearly that the nucleoli in *Zea* are formed by a definite heterochromatic region which she called the nucleolar-organizing body, and that the constriction thread was a result of nucleolar formation.

Matsuura (1938) was unable to find satellite or secondary constrictions in *Trillium kamschaticum* and reported that the nucleoli were organized at the end of the short arm of chromosome E and at both ends of chromosome A. He designated the nucleolar chromosomes of the type he found in *Paris* as the interstitial type and the *Trillium* chromosomes as the terminal type. He further stated that he could find no heterochromatic body similar to the nucleolar-organizing body of *Zea* in either *Paris* or *Trillium*.

In *Medeola* a large heterochromatic region similar to the nucleolar-organizer in *Zea* is always seen associated with the nucleolus in mid-prophase of meiosis, although it is hidden by the euchromatin in all the more condensed stages. McClintock showed that neither the relative size of the organizers within a cell nor the relative size between different strains was important. Therefore, it seems possible that nucleolar-organizers may vary in size, even between closely related forms, and those in *Paris* and *Trillium* may be very small.

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SECONDARY SPORES IN THE MYCELIUM OF THE CULTIVATED MUSHROOM, *PSALLIOTA CAMPESTRIS* FR.¹

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THE WRITER has had occasion, in connection with the commercial manufacture of spawn, to keep on hand a number of cultures of the cultivated mushroom, *Psalliota campestris*. Many of these cultures were transferred only at intervals of about six months. Following the transfer of old agar tube cultures, mycelia have occasionally been observed arising near the bottom of the slant independent of the piece of inoculum and having no observable connection with it. When transfers were made to Petri dishes from old agar tube cultures, mycelia occasionally arose which were distinctly separate from the piece of inoculum. This same phenomenon has also been observed by Dr. J. W. Sinden of the Pennsylvania State College, who has worked extensively with cultures of this organism. On the other hand, transfers made from relatively young cultures of *P. campestris* have never been accompanied by the development of mycelium in any region except that in direct contact with the inoculum.

On the basis of these observations, covering a period of about two years, it seemed clear that hyphal elements of some kind, which were being produced in old cultures, became detached during the transfer and unexpectedly initiated mycelia. To the writer's knowledge, no hyphal structures of the character and behavior mentioned above have been previously described in the mycelium of *P. campestris* (Colson, 1935; Hein, 1930; Sass, 1928). These observations led to further detailed studies on the mycelia of old cultures of this organism. A large number of old cultures on potato-dextrose and malt agars have been examined. Hyphal elements which resemble chlamydospores have been consistently observed in cultures of more than three months' duration. Chlamydospore-like cells have not been observed in young cultures of *P. campestris*. It seems likely that they have not been previously described, because old cultures were probably not studied in detail.

CHLAMYDOSPORE-LIKE CELLS IN VARIETIES OF *P. CAMPESTRIS*.—Chlamydospore-like bodies were first observed in the mycelia of the variety known as "snow-white." This variety is the one now almost exclusively cultivated in the eastern United States. In previous years other varieties of mushrooms distinctly different from the "snow-white" have been cultivated. They have all been considered varieties or forms of the cultivated mushroom, but occasionally attempts have been made to give them specific status. They include a great diversity of forms, ranging from those having a thick stem with a darkbrown, scaly cap to those which are light-cream, thin-stemmed,

and without floccose scales on the pileus. In the trade these varieties are familiarly known as the "big brown" and the "cream." Old cultures of the brown and cream varieties may form chlamydospore-like bodies as freely as the snow-white. Therefore, most of this study was carried out with the snow-white, since it appears to be representative of the species.

The occurrence of chlamydospore-like elements in the cultivated two-spored varieties of *P. campestris* affords an opportunity to examine further their relationship to the wild four-spored variety. Miss Colson (1935) has shown that, in general, the mycelial features of the wild variety correspond to those of the cultivated types. The quadrisporous wild variety differs from the bisporous cultivated one in habitat, distribution, and physiological behavior. No one has succeeded, so far as the writer is aware, in growing the wild variety in beds of composted manure. The distinction between these two forms is further emphasized by the fact that none of the chlamydospore-like bodies was observed in old cultures of the wild type. Owing to the conspicuous formation of chlamydospore-like bodies, the mature mycelium of the cultivated form presents an altogether different aspect from that of the wild type. This distinction further establishes the independent status of the two forms, a point which is frequently overlooked, particularly in popular works.

CHLAMYDOSPORE-LIKE CELLS IN THE MYCELIA OF FERTILE RACES.—The snow-white strain of *P. campestris* comprises an indeterminate number of races differing in rate of growth, ability to sector, cultural appearance, productivity, etc. Commercial spawn makers propagate desirable races from basidiospores or by culturing a piece of tissue from the sporophore. Cultures obtained by either of these methods are generally fertile, that is, they are capable of producing mushrooms. Studies of many fertile races showed that all of them produced chlamydospore-like cells in old cultures, and, as a matter of fact, microscopically they were all very similar.

The cells of the young hyphae, which are rather long and narrow and anastomose freely, are organized into highly branched filaments. Under the low-power objective it is difficult to distinguish the septum between adjoining cells. In old hyphae, on the other hand, the cells are very much enlarged transversely and considerably reduced in length. The hyphae appear to be fragmented into series of conspicuous chain-like cells. As a result of the thickening and rounding off of the end walls, the cells assume an individual character and can easily be seen under the low-power objective. The size and disposition of such cells, which are here regarded as secondary spores, readily distinguish them from the narrow, elongated cells of the young mycelium. For the sake of convenience, these cells which are uneven in size and

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shape may be called chlamydospores. Unfortunately, the term chlamydospore has been rather loosely used. Clearly they are not oidia as Brefeld (1877) and Brodie (1936) used the term. Biggs (1937) has illustrated swollen and bulbil-like cells in the mycelium of *Corticium coronilla* which are rather thick walled and which, in her opinion, resemble chlamydospores more than conidia. These cells are somewhat similar to those described in this study in the mycelium of *P. campestris*. In this discussion, chlamydospores are regarded simply as transformed vegetative cells which are capable of giving rise to new mycelia.

Ordinarily, young cells are rarely more than $4\ \mu$ in diameter and may attain a length of $45\ \mu$ or more. The chlamydospores, on the other hand, were rarely less than $8\ \mu$ in diameter, and rarely exceeded $25\ \mu$ in length. An average chlamydospore is about $10\ \mu$ by $14\ \mu$. Reduction in the length of each cell is accomplished by means of septa which partition the young cells as they mature. The chlamydospores are ovoid, globose, rectangular, elliptical, and show eccentric modifications of these shapes. The swelling of the cells as they mature presumably indicates an accumulation of food materials. This is evidenced by the fact that the cytoplasm of chlamydospores appears to be loaded with granular material and is much denser than the cytoplasm of young cells. Not all the cells in an old mycelium enlarge in this fashion, so that occasionally a long narrow cell appears in a chain of chlamydospores. The cells deep down in the agar medium were never observed to be converted into chlamydospores. As a result of random septation and enlargement, chlamydospores may be terminal, intercalary or, as is more frequently the case, in long chains. Some of the spores showed a very conspicuous thickening of the walls and appeared to be easily separable from contiguous cells. Most of the chlamydospores developed in hyphae which were imbedded in the uppermost part of the agar medium. Segmentation of the hyphae into clearly dissociable units did not occur to the extent that the chlamydospores were deciduous of their own accord as, for instance, is the case with secondary spores of species of *Mucor*, *Saprolegnia*, *Coprinus*, etc. In a great many cases the cell walls were not conspicuously thickened and the cells in a chain did not appear to be readily separable. All stages in the formation of chlamydospores were observed (fig. 7 and 8). Frequently swollen cells which were to undergo septation showed a pinching or constricting, so that they appeared somewhat peanut-shaped. Hein (1930) noted large irregular cells in the mycelial strands of *P. campestris*. These have nothing in common with chlamydospores. They are thin-walled, do not contain a dense cytoplasm and frequently have their end walls dissolved to form long tubes. In addition, they occur only in the mycelial strand in which they apparently serve as conducting elements.

Chlamydospores have a pronounced avidity for the stains used in studying them. Cotton blue, safranin, and haematoxylin bring them out clearly. Occasionally groups of cells appear which do not take the

stains at all and which are apparently devoid of protoplasmic contents (fig. 5). Ordinarily chlamydospores contain large quantities of granules of different sizes and shapes dispersed unevenly throughout the cytoplasm.

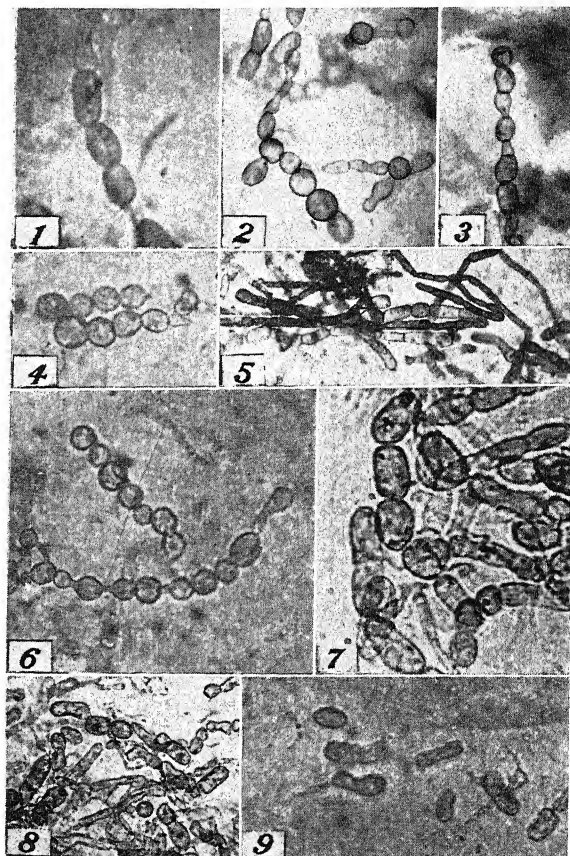


Fig. 1-9. Secondary spores in fertile and sterile races of *P. campestris*.—Fig. 1. Three chlamydospore-like cells in a fertile race. Note empty cell at each end. $\times 520$.—Fig. 2. Chain of secondary spores in a sterile race. $\times 450$.—Fig. 3. Variations in size and shape of spore-like cells in a sterile race. $\times 450$.—Fig. 4. Formation of secondary spores in a sterile race by constriction or "furrowing." $\times 450$.—Fig. 5. Cell types in a fertile race. Note chains of apparently empty cells. $\times 450$.—Fig. 6. Stages of spore formation in a sterile race. $\times 450$.—Fig. 7. Variations in size and shape of chlamydospore-like cells in a fertile race. $\times 550$.—Fig. 8. Cell types in a fertile race. $\times 400$.—Fig. 9. Single cells of a fertile race separated by crushing on a glass slide. $\times 450$.

For making temporary mounts, agar blocks containing the mycelium were stained in aqueous safranin and cotton blue and crushed under a cover glass in lacto-phenol. In safranin mounts of young mycelia, hemispherical pads were occasionally observed on both sides of a septum. These pads have been noted by previous investigators of this species and other Hymenomycetes (Colson, 1935; Hein, 1930; Sass, 1928). However, in cells which are being converted into chlamydospores and which have their end walls rounded off, such pads were never ob-

served. Presumably, these pads in young cells are connected by a thin strand of protoplasm which passes through a pore in the septum. The fact that they were not observed in the chlamydospores further emphasizes the independent character of such cells.

In studying the nuclei it was not necessary to employ the paraffin microtome technique. Thin agar slices were fixed and stained in Heidenhain's haematoxylin. After destaining the slices were mounted in glycerin jelly and crushed under a cover glass. This type of mount gave a better idea of the three dimensional character of the mycelium.

Chlamydospores are multinucleate and generally contain three to six nuclei irregularly disposed. Cells of the young mycelium are also multinucleate but as a rule contain more than six nuclei in a cell (Colson, 1935; Hirmer, 1920; Sass, 1928). The larger chlamydospores sometimes contained more than six nuclei. The nuclei were not disposed in pairs as in the secondary mycelia of many Hymenomycetes. Infrequently a cell was observed which seemed to have only one or two nuclei, but imperfections in the staining process and the extremely small size of the nuclei prejudiced the reliability of such counts. Dividing nuclei were not observed in mature cells. In the Hymenomycetes, so far as known, nuclear divisions generally occur in the tip cells of the hyphae, where growth is taking place. Hirmer (1920) has shown that this is also true for *Psalliotia campestris*, and the writer has confirmed this by staining mycelia grown on glass slides. Chlamydospores contained, as pointed out above, three to six nuclei, whereas younger cells contained more nuclei. The reduction in the number of nuclei in old cells may be explained by the fact that septa subsequently form which partition the mother cell into two or three daughter cells. Older cells are, of course, much shorter in length as a result of this partitioning. Hirmer (1920) noted that cells of the hyphae in older cultures of *Psalliotia perrara* contained fewer nuclei than cells of young cultures. Since he observed that nuclear behavior in *P. perrara* corresponds closely to that in *P. campestris*, it seems evident that septation is responsible in both of these species for the reduced number of nuclei in old cells. This type of behavior is not at all frequent in the Hymenomycetes. Usually a dikaryotic mycelium, for instance, which contains two nuclei in every cell, suffers no further changes in the nuclear content of each cell as the mycelium ages. In most Hymenomycetes the distribution of daughter nuclei in the tip cells is associated with the formation of a septum, so that the daughter nuclei are allocated evenly to the two cells which result (Buller, 1941). Thus, septum formation is always associated with nuclear division in the tip cells. In this case, however, the formation of septa which occurs in older cells as a process independent of cell formation, appears at the tip to be not at all associated with nuclear division.

It has been argued that chlamydospore formation is purely a laboratory phenomenon which does not

occur under field conditions. In many species it seems to be associated with the drying up and staling of the medium. In *P. campestris* there is no evidence that chlamydospores occur anywhere other than in old agar cultures. Attempts to find them in mycelia grown on manure and rye grains have been unsuccessful, even after the cultures were exposed to low temperatures for months. At any rate, the fact that chlamydospores develop in old cultures of *P. campestris* emphasizes further the latent possibilities inherent in a mycelium for acquiring new growth habits under special environmental conditions.

The formation of chlamydospores is most abundant at and just below the surface of the agar. The aerial part of the mycelium is apparently not converted into chlamydospores. The mycelium next to the inoculum piece produces the greatest number of chlamydospores, since it is the oldest part of the culture. For the most part chlamydospores are imbedded in the agar and are not free for dissemination, as is the case with most secondary spores. The appearance of errant mycelia when cultures were transferred is attributed to the dislodging of chlamydospores lying at the surface of the medium. Since for the most part they tend to remain in place, the phenomenon is only occasional. As will be described later they can be separated by mechanical means, though it must be emphasized that normally they do not appear to be deciduous of their own accord. Apparently nutrient deficiency encourages their formation. In cultures grown on potato extract without dextrose they begin to form earlier and are more abundant.

OCCURRENCE OF SECONDARY SPORES IN STERILE RACES.—As a rule monosporous cultures are fertile (Lambert, 1938). Sinden (1937) has pointed out, however, that cultures from single basidiospores are sometimes sterile, that is, spawn made from such cultures is not capable of producing sporophores. The mycelia of sterile races derived from single spores show a great many variations from the normal fertile types. As a matter of fact, every manner of physiological and morphological variation can be discovered in sterile races. A description of these variations is not pertinent here. Sterile races are included in this discussion because it is precisely in some of these that chlamydospore production is most pronounced. Moreover, chlamydospore production in some of these races is not a result of the aging of the culture but seems to be a function of growing mycelium.

Fifteen sterile races have been studied.² Some of these are so divergent from the normal type that they could never be identified as *P. campestris* if their origin were not known. In four races chlamydospores were produced in great abundance and with striking regularity in shape. In these the youngest hyphae show frequent swellings and constrictions in preparation for spore formation. Only the mycelia of these four races will be described. The spherical cells figured by Biggs (1937) in her group IV isolates of

² Seven of these races were obtained from J. W. Sinden of the Pennsylvania State College.

Corticium coronilla are very similar to those which occur in these sterile races of *P. campestris*.

In the fertile races, chlamydospores are formed by the production of septa which partition young cells. The resultant cells swell up, round off at the margins, and become chlamydospores. In the sterile races, on the other hand, spores are not cut out by septa formation. Young hyphae swell up in such a way that in outline they are very undulating. Swollen regions alternate in a regular series with regions of much lesser diameter. Ultimately, as a result of the continued swelling, the sides of the hyphae where no swelling occurs are brought into contact and spores are pinched off. It is possible that a process of constriction also aids in pinching off spores in this manner. The spores which result are consistently rounder and more uniform than those produced in fertile races (fig. 2 and 6). In contrast to this method cells can frequently be observed which are giving rise to buds. Buds may apparently arise anywhere along the periphery of a wall, and so may be terminal, lateral, or eccentric. Large and small cells alike may give rise to one or more buds which are finally separated from the mother cell by closure of the walls. In old cultures practically the entire mycelium is converted into chlamydospores. Generally the secondary spores of the sterile races are not as thick walled as are those which occur in fertile strains.

In stained mounts the cytoplasm appears very smooth and finely granular. No large granules or conspicuous inclusions occur nor does the cytoplasm appear to be as dense as in the fertile races. In studying nuclei great difficulty was encountered in getting them to stain properly. In general two to four nuclei were observed in each cell. Larger cells were observed in which as many as seven nuclei were counted. Very small spores, which are not infrequent in the four sterile races under discussion, often appeared to be uninucleated. The trapping of nuclei in spores follows the same general pattern as in the fertile strains. Young cells contain many more nuclei than older cells which have been cut out as chlamydospores. It is of interest to note that these strains which are so divergent from the normal fertile types are a cogent proof of the amount of novelty which can be secured with single spore isolations.

ISOLATION OF SINGLE CHLAMYDOSPORES IN FERTILE RACES.—The existence of chlamydospores in the mycelium of *P. campestris* offers an opportunity to examine their sexual and genetical nature. The mycelium of *P. campestris* does not exhibit clamp connections nor are the cells binucleate as is the case in the secondary mycelium of most of the Hymenomycetes (Buller, 1941). Ordinarily the basidiospores of *P. campestris* are initially bi-nucleate and single basidiospores give rise to a bisexual mycelium which is fertile. In the multinucleated cells of the mycelium the nuclei do not seem to be disposed in pairs so that there are no evident forces operating to hold nuclei of opposite sex together (Colson, 1935; Hirmer, 1920; Sass, 1928). The disposition of nuclei in pairs first becomes evident in the subhymenium, where it

is possible to observe as many as three pairs of nuclei in one cell. In view of the fact that chlamydospores are cut out by a process of random septation, it certainly seems possible that a chlamydospore might be produced which contained nuclei of only one sex. Unisexual spores are sometimes produced on a bisexual mycelium in certain Ascomycetes and Hymenomycetes. The conidia produced on the bisexual mycelium of *Neurospora tetrasperma* are frequently unisexual (Dodge, 1928). A unisexual conidium may contain more than one nucleus provided the nuclei are all of the same sex. This phenomenon happens as a result of random trapping of nuclei during spore formation. In *Collybia velutipes* the oidia produced on the bisexual secondary mycelium are regularly uni-nucleated (Brodie, 1936). In this way unisexual oidia are produced on a bisexual mycelium. The following experiment was designed to determine whether this phenomenon, which has been called deploidization by Buller (1941), may occur in the mycelium of *P. campestris* when chlamydospores develop.

Since chlamydospores are imbedded in the agar, special techniques must be employed to make single spore isolations. Considerable difficulty was encountered because the cells tended to stick together in groups. Agar blocks containing the spores were put into two per cent dextrose solution for two days and then crushed between glass slides. The soft mass was then washed off into a test tube by means of a pipette. The tube was agitated vigorously and the contents sprayed over the surface of a series of Petri dishes containing potato-dextrose agar. Microscopic examination showed that in a few instances single chlamydospores had been separated by the treatment. A week later spores which showed signs of developing were picked off with a needle. Twelve single-spore cultures were finally obtained.

No cytological or morphological differences could be observed in these twelve cultures. In each case the typical multinucleated mycelium of *P. campestris* appeared. The parent culture was a single basidiospore race, and thus had genetic status. Twelve bottles of rye spawn were made from the twelve cultures. One bottle of spawn was used to plant forty-five square feet of bed space in a commercial mushroom house so that in all 540 square feet were spawned. Mushrooms began to appear on all twelve plots in about six weeks. The number of pound baskets picked from each plot was recorded. The plots were kept in bearing for three months. The approximate yield of each plot fell rather close to one and a half pounds per square foot. It was evident that there were not significant variations of any character in any of the twelve samples. It seemed that no dissociations from the parent culture had occurred as a result of a single cell isolations. Apparently any cell in the culture is endowed with capacities which identify it with all other cells in the same mycelium regardless of size, shape, and nuclear content. Since all the cultures were fertile, it may be assumed that each chlamydospore contained at least two nuclei of

opposite sex. If unisexual cells do occur, they are rather infrequent. Even though the nuclei are not disposed in pairs, it seems evident that they are distributed in such a way that each cell receives at least two which are of opposite sex. Perhaps the multinucleate condition may, on the basis of these observations, be considered as a protection against the segregation of nuclei into unisexual spores. It is worth noting that in most genetical studies with single spore cultures, the investigations have very largely been carried out in Petri-dish cultures. In this instance, an opportunity to study hundreds of sporophores under field conditions was afforded. Variations affecting the mushrooms would most certainly have been observed.

SUMMARY

Chlamydospore-like cells have been described in varieties and races of the cultivated mushroom, *Psalliota campestris* Fr.

The brown and white varieties resemble each other in their capacity to produce chlamydospores. The

wild four-spored variety does not exhibit this capacity in agar cultures.

Fertile races produce secondary spores which are very variable in size and shape and which tend to be linear rather than round. The cytoplasm of such spores is dense and loaded with granules. Sterile races produce secondary spores which are uniformly spherical and which do not contain a dense cytoplasm. In addition, the young mycelia of certain sterile races produce chlamydospores in contrast to the fertile races in which chlamydospores are produced only in mature mycelia.

Chlamydospores in sterile and fertile races are multinucleate. Twelve single chlamydospores were isolated from a fertile race and mushrooms were obtained from each of the cultures made from these spores. No variations in any of the twelve cultures have been observed.

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INHERITANCE OF SMUT RESISTANCE IN HYBRIDS OF NAVARRO OATS¹

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THE NAVARRO oat variety has exhibited a remarkable resistance to many races of loose and covered smut (Reed, 1940). It has been tested with nineteen races of loose smut (*Ustilago avenae* (Pers.) Jens.) and fourteen of the covered (*U. levis* (Kell. and Sw.) Magn.), and with additional collections of both smuts, most of which probably belong to the races differentiated. No smutted plants of Navarro have been recorded except in one case where one infected plant, possibly a stray of some other variety, was observed.

The first Navarro plant was found in an oat field in Navarro County, Texas, about 1919, nothing definite being known concerning its origin (Stanton, 1933). It may have arisen as a hybrid between a variety of red oats (*Avena byzantina* C. Koch) and the common oat (*A. sativa* L.). Navarro has close affinities with the former collective species and is ordi-

narily classified as a member of the red oat group. It is very distinctive in several characters. It is an early, semi-erect variety and, in the later stages of growth and maturity, has a well defined purplish bloom. The panicles are compact with large, plump, three-grained spikelets. It does not rank high from the standpoint of yield or cold resistance. Further, it is very susceptible to rusts. Its chief agronomic value is due to its smut resistance, and it has been used extensively in oat breeding investigations.

The high degree of resistance of Navarro makes it interesting from the standpoint of the genetics of resistance to smuts. Accordingly, it has been crossed with other varieties, and the reaction of the hybrids to definite races of both loose and covered smuts has been determined. Successful crosses between Navarro and Hull-less, Black Mesdag, and Gothland were made in 1936. The F₁ plants were grown in 1937, the F₂ plants in 1938 and later years, and the F₃ generations in 1939, 1940, and 1941.

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The methods previously described were employed (Reed, 1941). Usually, the inoculated F_2 plants were grown to maturity in the greenhouse, although some were planted in the field. Most of the data on the third generation progenies were obtained in the field, although some families were grown under greenhouse conditions. The seed, inoculated by dusting with dry chlamydospores, was germinated in sand with a moisture content of about 20 per cent of its water-holding capacity and at a temperature of approximately 20°C . The seedlings usually emerged about the fourth day and were ready for transplanting in six to eight days.

The data for the reaction of the parental varieties to the smut races used for inoculation are recorded in table 1 and for the second and third generations of the hybrids in table 2. In the latter table the distribution of the third generation progenies, based upon the percentage of infection, is given. The F_3 generation progenies were inoculated with different races of smuts, the seed of the F_2 plants being divided into sets, each set being inoculated with one of the races.

In the discussion, the third generation progenies are grouped as resistant, segregating, and susceptible. In resistant progenies, no infected plants occur. It is not easy to separate the segregating and susceptible progenies in the hybrids where two or more factors are involved. The classification of segregating progenies as giving less than 50 per cent infection, while the susceptible progenies give more than 50 per cent of infected plants, is fairly satisfactory where a single factor for resistance is concerned. In cases where two or more factors are involved this

same grouping has some convenience in presenting the data.

HYBRID 90. NAVARRO \times HULL-LESS.—Four crosses between Navarro and Hull-less were made, and separate sets of seeds of the second and third generations were inoculated with three races of *Ustilago avenae* and two of *U. levis*. Navarro is resistant to all five of these races, while Hull-less is susceptible to four and resistant to one.

Results with *Ustilago avenae*, Race 1.—In the experiments with this race of smut 241 plants of Hull-less were inoculated, of which 222 (92.1 per cent) were infected, the results in the different experiments ranging from 80.9 to 100 per cent. In contrast, the 241 inoculated plants of Navarro gave no infection.

There were 74 F_2 plants inoculated with race 1 of the loose smut and 7 (9.4 per cent) were infected. These data may indicate that there is a two-factor difference in the inheritance of resistance to this race of smut. On this basis, 6.25 per cent of the F_2 plants should be infected, or 5 out of the 74 inoculated.

The data for the F_3 generation are in harmony with the two-factor explanation, 265 progenies being grown, with the following results:

	Resistant	Segregating	Susceptible
Observed	116	127	22
Expected (7:8:1)	116	132.5	16.5

It is, of course, not at all easy to separate sharply the segregating from the susceptible group. How-

TABLE 1. Reaction of parental varieties of hybrids to the specialized races of *Ustilago avenae* and *U. levis*.

Varieties	Seed No.	No. of plants	No. infect.	Per cent infect.
<i>Ustilago avenae</i> —Race 1:				
Gothland	152	209	205	98.0
Hull-less	30	241	222	92.1
Navarro	939	241	0	0
<i>Ustilago avenae</i> —Race 12:				
Black Mesdag	70	174	0	0
Hull-less	30	397	325	81.8
Navarro	939	506	0	0
<i>Ustilago avenae</i> —Race 10:				
Black Mesdag	70	37	0	0
Gothland	152	115	1	0.8
Hull-less	30	53	0	0
Navarro	939	125	0	0
<i>Ustilago levis</i> —Race 1:				
Hull-less	30	208	174	83.6
Navarro	939	226	0	0
<i>Ustilago levis</i> —Race 7:				
Black Mesdag	70	287	193	67.2
Hull-less	30	202	167	82.6
Navarro	939	338	0	0
<i>Ustilago levis</i> —Race 9:				
Black Mesdag	70	81	71	87.6
Navarro	939	66	0	0

TABLE 2. Distribution of F_3 progenies of oat hybrids, based on the percentage of plants infected with definite races of *Ustilago avenae* and *U. levis*.

Race of smut	Data for the F ₂ generation			F ₃ progenies in indicated class center for percentage of infection											Summary for the F ₃ progenies				
	Plants grown	No. infect.	Per cent infect.	Resistant	Segregating					Susceptible					No. grown	Per cent infect.			
					0	5	15	25	35	45	55	65	75	85			95	100	
Hybrid. 90. Navarro Hull-less	A- 1	74	7	9.4	116	57	34	19	13	4	4	3	4	6	2	3	265	149	56.2
	I- 1	70	4	5.7	148	54	27	16	3	3	1	1	5	5	1	1	265	117	44.1
	I- 7	132	4	3.0	124	40	30	10	5	6	4	0	0	4	1	2	226	102	45.1
	A-10	17	0	0	20	3	1	0	0	0	1	0	0	0	0	0	25	5	20.0
	A-12	121	62	51.2	26	16	18	14	16	22	20	22	27	19	14	11	225	199	88.4
Hybrid 91. Navarro Black Mesdag	L- 7	62	3	4.8	97	23	8	1	0	0	0	0	0	1	0	0	130	33	25.3
	I- 9	—	—	—	130	9	4	0	0	0	2	0	0	0	0	1	146	16	10.9
	A-12	63	3	4.7	81	30	8	4	2	2	1	1	1	0	0	0	130	49	37.6
	A-10	—	—	—	25	0	0	0	0	0	0	0	0	0	0	0	25	0	0
	A- 1	67	5	7.4	78	37	22	13	6	2	1	1	0	3	2	0	165	87	52.7
Hybrid 92. Navarro Gothland	A-10	17	0	0	23	1	0	1	0	0	0	0	0	0	0	0	25	2	8.0

ever, if these two groups are combined, we find that 56.25 per cent of the progenies should contain smutted plants and a percentage of 56.22 was actually obtained in the experiments.

Results with Ustilago levis, Race 1.—In the experiments with this smut 208 plants of Hull-less were inoculated, of which 174 (83.6 per cent) were infected, the percentage varying from 58.3 to 97.2; 226 plants of Navarro were inoculated and none was infected. In the F_2 generation 70 plants were inoculated and 4 (5.7 per cent) were infected. These data may indicate either a two- or three-factor difference, and the results for the third generation are necessary to determine which is the more probable explanation. On the basis of two factors, 6.25 per cent of the F_2 plants should be infected while, on the assumption of three factors, the percentage of infection should be 1.55. There were 265 F_3 progenies grown, with the following results:

	Resistant	Segregating	Susceptible
Observed	148	103	14
Expected (37:26:1)	153	108	4

The distribution of the progenies is in line with the three-factor assumption and quite different from what would be obtained if only two factors for resistance were involved.

Results with Ustilago levis, Race 7.—There were 202 plants of Hull-less inoculated with this race of covered smut and 167 (82.6 per cent) were infected. Usually, the percentage of infection in the different experiments was high; in one test, however, only 45 per cent of the inoculated plants were smutted. In contrast, 338 plants of Navarro were inoculated and none was infected.

There were 132 F_2 plants of the hybrid inoculated and 4 (3 per cent) were infected. These results may indicate that three different factors for resistance are involved, and this assumption implies that, out of a total of 132 plants, 130 should be resistant and 2 (1.55 per cent) infected. In the third generation, 226 progenies were grown, the results secured being as follows:

	Resistant	Segregating	Susceptible
Observed	124	91	11
Expected (37:26:1)	130	92	3

The results secured correspond quite well with those obtained with the F_2 generation and indicate that three distinct factors for resistance are involved. The combined groups of segregating and susceptible constitute 45.1 per cent of the progenies while, according to expectation, 42.17 per cent should be infected.

Results with Ustilago avenae, Race 12.—Hull-less is susceptible to this race of smut, 397 plants being inoculated and 325 (81.8 per cent) infected. Navarro, in contrast, is resistant, no infected plants being found in a total of 506 inoculated.

The results with both the second and third generations of this hybrid, inoculated with this race of smut, are in striking contrast to those obtained with the previous races. A total of 121 F_2 plants was inoculated and 62 (51.2 per cent) were infected. Altogether, 225 F_3 progenies were inoculated with this race and 26 were resistant, 86 segregating, and 113 susceptible. The results for both generations indicate a very different basis for the inheritance of smut resistance as compared with the F_2 and F_3 generations inoculated with Avenae-1, Levis-1, and Levis-7.

Results with Ustilago avenae, Race 10.—Both Navarro and Hull-less are resistant to the Red Rustproof race of loose smut, 125 plants of Navarro having been inoculated with various collections belonging to this race and none infected. In similar tests 53 plants of Hull-less have given negative results.

A very small number of F_2 plants was inoculated with one collection of the Red Rustproof race, none of them being infected. There were 25 F_3 progenies grown, inoculated with another collection of this same race of loose smut. Most of these progenies were resistant; three of them, however, contained one smutted plant, another progeny three smutted plants and, in another, slightly more than half of the plants were infected. It is evident that susceptible individuals may be obtained among third generation progenies, although both the original varieties are resistant.

HYBRID 91. NAVARRO \times BLACK MESDAG.—The second and third generations of this hybrid were inoculated with two races of covered smut and two of the loose.

Results with Ustilago levis, Race 7.—Black Mesdag is susceptible to this race of covered smut, 287 plants being inoculated, of which 193 (67.2 per cent) were infected. In two of the experiments rather low percentages of infection (40 and 43.7) were obtained; in the field in 1938 100 per cent of the inoculated plants were infected. In contrast to the results secured with Black Mesdag, Navarro proved resistant, 338 plants being inoculated and none infected. Altogether, 62 F_2 plants were inoculated and 3 (4.8 per cent) were infected. A total of 130 inoculated F_3 progenies was grown. The results indicate a much more complicated situation than a two- or even three-factor basis, and agree more closely with the assumption that at least five independent factors for resistance are involved. The correspondence between observed and expected results on this basis are as follows:

	Resistant	Segregating	Susceptible
Observed	97	32	1
Expected (781:242:1)	98	32	0

The occurrence of three infected F_2 plants in a population of 62 would not be expected, since only one smutted plant out of 1,023 should be found. A susceptible F_3 progeny in such a small population also would not be expected. The F_3 progeny classified as such, however, gave 83.3 per cent infection.

Results with Ustilago levis, Race 9.—Black Mesdag has proved to be susceptible to this particular race of covered smut. In the present experiments 81 plants were inoculated and 71 (87.6 per cent) were infected. Navarro, on the other hand, is resistant, 66 plants being inoculated and none infected. No F_2 plants were inoculated with this race. However, a total of 146 F_3 progenies was grown, and the data suggest a series of independent factors for resistance. Altogether, 130 progenies were resistant, 13 segregating, and 3 might be classified as susceptible. Ten of the F_3 progenies were retested, giving almost identical results as in the first experiment. The results do not fit very closely any particular formula, being nearest, perhaps, to an explanation on the basis of five factors.

Results with Ustilago avenae, Race 12.—Both the parental varieties are resistant to this race of loose smut and in these experiments 174 plants of Black Mesdag and 506 of Navarro were inoculated, no infected plants being observed in either variety.

There were 63 F_2 plants inoculated and 3 (4.7 per cent) were infected. A total of 130 F_3 progenies were grown, the results being as follows:

	Resistant	Segregating	Susceptible
Observed	81	46	3
Expected (37:26:1)	75.1	52.7	2

The F_2 data might be interpreted as either a two- or three-factor difference. On the other hand, the data for the F_3 progenies fit rather closely the assumption of three independent factors.

Results with Ustilago avenae, Race 10.—Both Black Mesdag and Navarro are resistant to the Red Rustproof race of loose smut, 37 plants of the former and 125 of the latter having been inoculated and none was infected. A series of 25 F_3 progenies of the hybrid was inoculated with a collection of this race. No infected plants were observed in any of the progenies.

HYBRID 92. NAVARRO \times GOTHLAND.—The second and third generations of this hybrid were inoculated with two races of loose smut.

Results with Ustilago avenae, Race 1.—In the course of the experiments 241 plants of Navarro were inoculated and none was infected, while 209 plants of Gothland were grown and 205 (98 per cent) were smutted.

There were 67 second generation plants inoculated with race 1 of the loose smut and of these 5 (7.4 per cent) were infected. These data suggest the presence of two factors for resistance since, on this assumption, the ratio between the normal and the

infected would be 15:1, or a total of 63 normal to 4 smutted plants. The data obtained for the third generation fit this interpretation, the results for 165 progenies grown being as follows:

	Resistant	Segregating	Susceptible
Observed	78	80	7
Expected (7:8:1)	72	83	10

Results with Ustilago avenae, Race 10.—Seventeen second generation plants were inoculated with Avenae 38, a collection which belongs to the Red Rustproof race. No infected plants were obtained. Along with these F_2 plants, 19 plants of Gothland and 17 of Navarro also gave negative results. Other collections of the Red Rustproof race have given similar results with these varieties.

In the same year 25 third-generation progenies, inoculated with another collection belonging to the Red Rustproof race of loose smut were grown. In one of the progenies a single infected plant out of a total of 23 was observed, and in another progeny 5 plants out of a total of 22. These results suggest that, although both parents are resistant, we may find, among a large number of F_2 plants, an occasional infected one and some F_3 progenies segregating or even susceptible to this particular race. Several independent factors would account for the resistance in the hybrid generations.

DISCUSSION.—The data for Hybrid 90 Navarro \times Hull-less, and Hybrid 92 Navarro \times Gothland, inoculated with race 1 of *Ustilago avenae*, fit quite well with the assumption of two independent factors for resistance. On this basis we would expect 6.25 per cent of the F_2 plants to be infected, and 56.25 per cent of the F_3 progenies would be classified as segregating and susceptible. In Hybrid 90, 7 F_2 plants out of 74 inoculated (9.4 per cent), and in Hybrid 92, 5 F_2 plants out of 67 (7.4 per cent) were infected. In the first hybrid 56.22 per cent of the F_3 progenies were classified as either segregating or susceptible; the progenies also separated into the two groups according to expectation. In Hybrid 92, 52.7 per cent of the F_3 progenies were included in the segregating and susceptible group, a close correspondence to the two-factor explanation.

The resistance of Hybrid 90 Navarro \times Hull-less to races 1 and 7 of *U. levis* appears to depend upon three independent factors; the data for the F_2 generation as well as for the F_3 closely fit this interpretation. In the F_2 generation we would expect 1.55 per cent of the plants to be infected. Actually, 5.7 per cent, or 4 F_2 plants out of 70 inoculated with race 1 were smutted and 3 per cent, or 4 F_2 plants out of 132 inoculated with race 7 were infected. In the third generation, 42.17 per cent of the progenies would be expected to be segregating or susceptible and, according to the data obtained, 44.1 per cent of the 265 F_3 progenies inoculated with race 1 and

45.1 per cent of the 226 F_3 progenies inoculated with race 7 belong to these two groups.

Resistance in Hybrid 91 Navarro \times Black Mesdag, inoculated with races 7 and 9 of the covered smut, doubtless rests on several factors, possibly as many as five. The data for the F_2 generation might suggest a three-factor difference, but the number of segregating and susceptible progenies in the F_3 generation is far too low for this explanation. The data for this hybrid, inoculated with A-12, indicate a three-factor situation.

The results with Hybrid 90 Navarro \times Hull-less, inoculated with race 12 of the loose smut, are very different from those just described. In the second generation, 121 plants were inoculated and 62 (51.2 per cent) were infected. The results indicate that susceptibility may be dominant over resistance; the percentage of infection, however, does not suggest any simple factor relation. Among the 225 F_3 progenies inoculated, only 26 were resistant, the remainder being classified as segregating or susceptible. Of these progenies, 86 were classified as segregating, giving less than 50 per cent infection, and 113 as susceptible, giving 50 to 100 per cent infection.

The results with this hybrid, inoculated with *Ustilago avenae*, race 12, are somewhat similar to data reported for other crosses (Reed, 1932). Gothland \times Hull-less gave a high percentage of infection of the F_2 plants inoculated with Levis 1. In the F_3 generation there was a very marked excess of segregating and susceptible progenies over the resistant. Similar results were obtained with Monarch \times Hull-less inoculated with Avenae 1. Schattenberg (1934) reports several cases where there was a very great shortage of resistant F_3 progenies and a corresponding large number of segregating and susceptible. Torrie (1939) obtained a similar result in the hybrid Iowa 444 \times Bond, securing 39 per cent infection of the F_2 plants and 24 resistant, 166 segregating, and 60 susceptible F_3 progenies out of a total of 250 grown.

No adequate explanation of these results has been given. Schattenberg (1934) assumes a single factor difference. Torrie (1939) suggests a 4:11:1 relation, including in the resistant group a large number of F_3 progenies which give low percentages of infection; he also assumes a factor for susceptibility as epistatic to a factor for resistance.

Marcy (1937) obtained somewhat similar results in sorghum hybrids between Feterita and other varieties susceptible to *Sphacelotheca sorghi* (Link) Clinton. She suggests the interaction of a factor for susceptibility (S) in the susceptible varieties, and a factor for resistance (B) brought in by Feterita. Under environal conditions highly favorable for infection, S has been demonstrated to be epistatic to B for segregation in the F_2 generation, giving approximately the ratio of 13 infected to 3 normal plants. Under conditions less favorable for infection, the F_2 ratios were nearly reversed, approaching 3 resistant to 1 susceptible. She concluded that hybrid plants

containing both the S and B factors were unstable in their infection reactions, and that the epistasis of S over B, or B over S, was determined by environal conditions during the germination period.

Many oat crosses have been reported in which the data for the F_2 and F_3 generations indicate a single factor for resistance. The data published for hybrids of Hull-less \times Black Mesdag, Silvermine \times Black Mesdag, and Early Champion \times Black Mesdag, inoculated with Avenae race 1 and Levis race 1, give results indicating a single factor for resistance (Reed, 1928, 1934). Similar results have been obtained for Fulghum \times Black Mesdag inoculated by Avenae race 12 (Reed, 1935), Gothland \times Black Mesdag, and Danish Island \times Black Mesdag inoculated with Avenae race 1, Green Mountain \times Monarch inoculated with Avenae race 1 and Levis race 3 (Reed, 1941). Von Rosenstiel (1929) records data for four hybrids in which the explanation is based on a single factor. Coffman *et al.* (1931) offer this explanation for hybrids of Markton \times Early Champion.

Two factors for resistance are assumed for other types of oat crosses. This is the explanation for the hybrid (Victory \times "Hybrid") inoculated with *U. avenae* and *U. levis* (Welsh, 1931). Hybrids of Markton \times Aurora, Ligowa, Scottish Chief, Swedish Select, and Victory were also explained on the two-factor basis (Coffman *et al.*, 1931).

Three factors are the probable explanation for other oat crosses. Iogren \times Markton and Silvermine \times Markton, as recorded by Coffman *et al.* (1931); Markton \times Colorado 37 (Austin and Robertson, 1936); Fulghum \times Black Mesdag (Barney, 1924); von Lochows Gelbhafer \times Red Rustproof (Nicolaisen, 1931). Schattenberg (1934) gives data on a hybrid in which a three-factor explanation seems adequate. Stanton, Reed, and Coffman (1934) reported on certain hybrids which might be explained on this same basis—Monarch Selection \times Black Mesdag inoculated with Levis race 1, and Richland \times Markton, Markton \times Iogold, and Cornellian \times Markton, inoculated with Avenae race 1.

Finally, there are several cases where four or more factors are necessary for a satisfactory explanation—Red Rustproof \times Abundance and Black Tartarian, inoculated with *U. levis* (Gaines, 1925); Richland \times Markton, Markton \times Iogold, and Cornellian \times Markton, inoculated with Levis race 1, and Markton \times Black Mesdag inoculated with Avenae race 1 and Levis race 1 (Stanton, Reed, and Coffman, 1934).

Third generation progenies of all three hybrids were inoculated with the Red Rustproof race, Avenae 10. No infected plants were observed in Hybrid 91. In Hybrids 90 and 92 an occasional progeny contained 1 to 13 infected plants.

SUMMARY

The Navarro oat variety is resistant to all the known races of *Ustilago avenae* and *U. levis* with

which tests have been made. Its marked resistance has been utilized in oat-breeding investigations, and the variety is interesting for the analysis of the inheritance of resistance to smut.

Hybrids of Navarro and Hull-less inoculated with *U. avenae* race 1, based on F_2 and F_3 data, indicate a two-factor relation for inheritance of resistance. A three-factor difference is indicated for these hybrids when inoculated with *U. levis* race 1 and race 7.

The hybrids of Navarro and Hull-less, inoculated with *U. avenae* race 12 differ markedly from the results just mentioned. There is a high percentage of inoculated F_2 plants infected; in the F_3 generation there is a very small number of resistant progenies and a great excess of segregating and susceptible.

In hybrids of Navarro and Black Mesdag, five factors for the inheritance of resistance to *U. levis* races 7 and 9, and three factors for *U. avenae* race 12 are indicated.

In the hybrid of Navarro and Gothland, inoculated with *U. avenae* race 1, two factors are indicated.

All of the parental varieties, Navarro, Hull-less, Black Mesdag, and Gothland, are resistant to the Red Rustproof race of loose smut, Avenae 10. An occasional F_3 progeny of Navarro \times Hull-less and Navarro \times Gothland contains infected plants. No smutted plants were found in the F_3 progenies of Navarro \times Black Mesdag.

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CARBON DIOXIDE EVOLUTION DURING THE INDUCTION PERIOD OF PHOTOSYNTHESIS¹

James Franck

IN THEIR report of experiments on the quantum efficiency of photosynthesis, Emerson (1939) and Emerson and Lewis (1941) have described some transients in the exchange of CO₂ which occur at the beginning and end of an illumination period. These transients bear some resemblances to the previously known induction period for photosynthesis and to the pickup phenomenon discovered by McAlister (1937). The induction and pickup phenomena are relatively small effects and have generally been interpreted as perturbations in the photosynthetic process and have accordingly been incorporated in theories of the kinetics of photosynthesis. The transients observed by Emerson and Lewis are under proper circumstances large effects: they report that at the beginning of illumination an "outburst" of CO₂ occurs, and that for a few minutes the rate of CO₂ evolution may be ten times the rate of photosynthesis. These phenomena, they believe, suggest that the plant contains a "reservoir" for CO₂ which is emptied rapidly by a photochemical reaction and is slowly refilled during a dark period.

Emerson and Lewis doubt that there is sufficient evidence for believing that the CO₂ reservoir has any direct connection with the photosynthetic machinery of the plant. They also suggest that the induction and pickup phenomena of carbon dioxide may be simply somewhat subdued manifestations of the reservoir. Consequently, they are led to question their significance for the photosynthetic theory. These criticisms are directed particularly at the induction and pickup as observed by McAlister (1937), by Aufdemgarte (1939), and by McAlister and Myers (1940), since their measurements are confined to the exchange of CO₂, but, if justified, would also invalidate the theory of the induction period developed by Franck, French, and Puck (1941) which is based on the same and similar measurements and on correlated anomalies of the chlorophyll fluorescence.

I feel unable to concur in this scepticism regarding the value of these studies of induction phenomena in

photosynthesis. It seems to me rather that one may regard Emerson and Lewis' observations as contributing further evidence in support of our interpretation of the induction period.

First, we shall give our objections to the explanation which Emerson and Lewis propose for the outburst. The facts observed by these authors and which have to be explained are the following: at all intensities the CO₂ outburst² occurs during the first two minutes of irradiation. In the region of small light intensities its rate is proportional to the light energy absorbed and its quantum yield is about one. A transition from lower to higher intensities produces a new outburst even after a long irradiation at the lower intensity. The rate of O₂ production (according to the curves published) is negligible during the CO₂ outburst but rises rapidly to its final value as the rate of the outburst declines.³ The final rate is proportional to the light intensity (as long as the latter is small compared with saturation intensities) and does not show the same dependence on the previous treatment of the algae as does the CO₂ outburst. Finally with continued irradiation, at all light intensities, the photosynthetic quotient approaches the value one.

If, as Emerson and Lewis assume, an unidentified substance X, which has no direct relation to photosynthesis, is the source of the CO₂ evolved, its concentration at the beginning of the irradiation period must be so great that it almost completely displaces from the chlorophyll those substances normally photosynthesized, for otherwise the quantum yield for the splitting off of CO₂ could not approach unity. On the other hand, it is evident that after an irradiation of two minutes X no longer competes with the substrate of photosynthesis, for then the rate of oxygen

² The term CO₂ outburst refers only to the strong main anomaly in the rate curve of the CO₂ exchange. A discussion of the small irregularities which follow the main maximum will be found further in the discussion.

³ One has to bear in mind, though, that according to Emerson and Lewis not too much importance should be attached to the precise shape of the rate curves of the O₂ production.

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production is normal at all light intensities. Consequently, we should conclude that after two minutes of irradiation, regardless of the intensity, the concentration of X is much smaller than that of the substrate of photosynthesis. But this conclusion is contradicted by the fact that a transition to a higher intensity produces a new outburst, which could occur only if X were still present.

One might attempt to avoid this contradiction by assuming that a small part of the chlorophyll is separated from the remainder and that the bulk of the chlorophyll is responsible only for photosynthesis while the smaller portion sensitizes the splitting of CO_2 from X. The quantum yield of about one must then be explained by a chain reaction. But a chain is very implausible for this type of reaction. Photooxidation chains are excluded, since the corresponding oxygen uptake is not observed, and other chains involving radicals as chain carriers and delivering CO_2 as the end product are difficult to imagine. The other type of chain, in which the energy liberated in one link of the chain activates the next one until the proper length is reached, seems to be excluded since such chains do not occur in a condensed system in which the energy is dissipated very quickly. In addition this hypothesis fails to explain the lack of oxygen production during the outburst, and requires additional assumptions to explain why the reservoir partially drained by a weak illumination is not filled up again in the dark in about the same time needed by the outburst to drain it.

Hence, we discard the hypothesis that the substance X, from which CO_2 is produced during the outburst, is one which is not intimately connected with the photosynthetic apparatus.

We now present the evidence for regarding the CO_2 outburst as a special form of the short induction period which differs from the normal cases only because of the peculiar conditions resulting from the special treatment of the algae which make the outburst prominent. If we accept this point of view it becomes self-evident that the short induction period and the outburst show the same general behavior. The duration of the outburst is the same as that of the normal induction period; the time course of both phenomena is independent of light energy, and they both show the very specific quality of being excited stepwise if the light intensity is increased stepwise. Furthermore, from our theory of the induction period we can obtain a detailed understanding of the outburst. We shall not repeat here the whole theory of the induction period⁴ and its experimental basis, but merely recapitulate its main features and then apply them to the present problem.

⁴ The theory of the short induction period is presented in a paper of J. Franck, J. French and F. Puck, *Journal of Physical Chemistry*, 1941. The idea that the induction phenomena are caused by a temporary deficiency of the oxygen splitting catalyst on which the theory is based is taken from a paper of H. Gaffron, *Naturwissenschaften*, 1937. The theory of the general kinetics of photosynthesis is presented in a paper of J. Franck and K. F. Herzfeld, *Jour. of Phys. Chem.*, 1941.

According to the theory, all the anomalies of the photosynthetic gas exchange and of the chlorophyll fluorescence during the short induction period are caused by a temporary deficiency of the oxygen splitting catalyst (called catalyst C in our treatment of the kinetics of photosynthesis). Catalyst C is deactivated in the dark and becomes reactivated during the beginning of an illumination period by a reaction with the reduced photosynthetic product. The duration of the readjustment is independent of the light intensity because, to prevent a limitation at small light intensities, only a small proportion of catalyst C need be active, while at higher intensities a correspondingly higher proportion is required. The limited efficiency of catalyst C during the induction period causes an accumulation of the photoperoxides. The accumulated peroxides react, under the conditions prevailing in most of the cases, with a metabolic product and transform it to a partially oxidized product, which in its turn acts as a poison for other catalysts involved in photosynthesis. In this way, the total photosynthetic yield is quickly reduced to a very low value. The recovery of photosynthesis occurs on the reactivation of catalyst C and an oxidative removal of the poison; the time required for this is about two minutes. That the photosynthetic yield is abnormally low during the induction period does not mean, however, that the number of photochemical reactions is small. We suppose, on the basis of quite independent evidence (Franck and Herzfeld, 1941), that the freshly formed photoproducts are chemically unstable and fall back to their original state if they are not stabilized quickly by a chemical reaction in which a catalyst "B" is involved. Since catalyst B is especially susceptible to that poison produced during the induction period, most of the freshly formed photochemical products undergo back reactions during the induction period and dissipate as heat the energy obtained photochemically.

The energy liberated by each back reaction is considerable; back reactions to the state of a loosely bound compound are, therefore, likely to break up such products. This is the case with the lowest intermediate of photosynthesis, which is an organic molecule loosely connected with a CO_2 molecule. According to Kamen, Ruben and co-workers (1939 and 1940), it is a reversibly carboxylated product written conventionally as RCOOH . As the result of a back reaction, it dissociates into RH and CO_2 . Several quite different sets of observations can be explained by means of this CO_2 splitting process. Since the rate of back reactions is great during the induction period, the possibility of a CO_2 evolution during that period is an important point. It is questionable whether under normal conditions a CO_2 evolution actually occurs during the induction period. Since apparently the rate of O_2 production (Blinks and Skow, 1938) runs parallel to the rate of CO_2 uptake (McAllister, 1937; McAllister and Myers, 1940; Aufdemgarten, 1939), we concluded that the splitting off of CO_2 is normally compensated by the carboxylation reaction which takes place with the

help of a catalyst A which is present in abundance so that no outburst of CO_2 occurs. In other words the experiments carried so far seem to indicate that the oxidized metabolite does not act as a strong poison for catalyst A involved in the carboxylation reaction. According to our point of view, the experiments of Emerson and Lewis are simply observations of induction periods carried out under conditions such that the carboxylation reaction is temporarily unable to compensate the photochemical decarboxylation of RCOOH . The condition necessary is the presence of an unusually great amount of RCOOH . Its concentration must be great compared with that of the other intermediates of photosynthesis, otherwise the quantum yield for CO_2 liberation could not be about unity. All the light absorbed is diverted to RCOOH ; the other intermediates are crowded away from the chlorophyll. That favors, of course, the evolution of carbon dioxide, because now all back reactions occurring during the induction period will result in splitting off CO_2 , causing a yield about ten times greater than normal.

If this be true, we should expect a strong recarboxylation, that is, a strong carbon dioxide absorption immediately upon the end of the induction period. Catalyst A is certainly able at this moment to handle an amount of RH equal to the amount of RH liberated during the outburst in about one minute. (Its efficiency is known experimentally from the fact that it still is not limiting at the high rate of photosynthesis prevailing at light saturation.) Since such a period of an abnormally strong uptake of CO_2 does not appear in Emerson and Lewis' curves, we are forced to assume that not only RCOOH dissociates under the conditions prevailing during the outburst, but that the resulting RH is excluded from further participation in the carboxylation reaction. We believe that this elimination is caused by the unusually great momentary concentration of RH which is the result of the high rate of its liberation during the outburst. If RH is indeed a carbohydrate molecule, its excess might be removed by a polymerization reaction.

The time course of the CO_2 exchange during the illumination period after the expiration of the outburst is normal if one takes into account that, even after the outburst, some surplus of the concentration of RCOOH over that of the higher intermediates is still present.⁵

A few words may be added to explain the time

⁵ During the steady state of photosynthesis the concentration of RCOOH is just equal to that of the other intermediates, and this even distribution remains normally unaltered after dark pauses of about one-half hour. (This latter condition is not fulfilled under the special conditions prevailing in Emerson and Lewis' experiments as will be discussed below). Therefore, if photosynthesis is resumed, no irregularities in the rate curves are observed, which may be attributed to a redistribution of the concentrations of intermediates. If on the other hand, conditions are chosen such that an uneven distribution is produced, the expected irregularities (called long induction periods) occur in the rate curves and have a duration which is inversely proportional to the final rate of photosynthesis. The latter part of

course of the CO_2 exchange in the dark period following an exposure to light for about one-half hour. Emerson and Lewis observed that a slow uptake of carbon dioxide occurs, which is superimposed on the normal carbon dioxide production by respiration for about forty minutes. This uptake of CO_2 is only observable if enough oxygen is present to allow an undisturbed respiration of the algae. The CO_2 accumulated in that way is available for a new outburst if irradiation is resumed. This carbon dioxide uptake in the dark is an entirely separate phenomenon from the pickup of CO_2 as observed by McAllister and Myers (1940) and Aufdemgarten (1939). The normal pickup is a process in which RH, liberated by photosynthesis or by back reactions during the illumination period, combines with CO_2 in the dark directly after the light is turned off. Free RH is only available at that time if catalyst A was limiting during the irradiation period. In the present case catalyst A was not limiting in the last twenty-five minutes of the prolonged irradiation with weak light and, consequently, free RH should not be present at all. One has, therefore, to find another explanation for the slow uptake of CO_2 in the dark. It is very probably caused by a slow new formation of RH. The concentration of RH present at each moment is so small that only the carboxylation reaction which employs catalyst A is effective in removing RH. The side reaction mentioned above, wherein RH is eliminated if present in high concentrations, can be neglected. While it would be premature to speculate at this time about the nature of the process of the formation of RH, it seems significant that it is connected with the respiration process. The formation of RH by metabolic processes and also its consumption by the side reaction thus indicated by the observation of Emerson and Lewis will certainly become of importance in further studies of the carboxylation process which precedes photosynthesis.

SUMMARY

Emerson and Lewis observed that with algae under special conditions a carbon dioxide evolution occurs at the beginning of an illumination period. They are inclined to suppose that this process has no direct connection with photosynthesis. The present paper is intended to show that what Emerson and Lewis (1941) observed is a special case of the normal induction period, and its interpretation is closely related to that given by Franck, French, and Puck (1941) for other induction phenomena. From this the time course of the CO_2 exchange as observed by Emerson and Lewis (the second weak maximum of the curve followed by the slow transition to the steady state of CO_2 uptake) shows these typical anomalies. It is in accordance with expectation that such anomalies are absent in the rate curves for the O_2 production. The rate of O_2 evolution depends on the distribution of the concentrations of the hydrogenated and dehydrogenated hydrogen donors and not on the relative concentrations of the hydrogen acceptors (RCOOH and its partially reduced derivatives). Only great anomalies in the absolute concentration of the latter will influence the rate of the oxygen production in an indirect way.

point of view the carbon dioxide evolution is explained by back reactions and thus lends support to the principle advanced by Franck and Herzfeld (1941) that back reactions influence kinetics of

photosynthesis whenever catalytic limitations occur.

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AN ANALYSIS OF THE COMPARATIVE RATES OF CELL DIVISION IN VARIOUS PARTS OF THE DEVELOPING CUCURBIT OVARY¹

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THE DETERMINATION of comparative rates of cell division in different tissues or regions is an important problem in plant development but is difficult to accomplish by direct mitotic counts. Another method of attacking it is to determine the increase in cell number for given tissues over a given period. The young cucurbit ovary is particularly good material for such a study. Its various regions are sharply delimited, and their volumes can be measured with fair accuracy. Average cell volume can also be measured for each, and if region volume is divided by cell volume, the approximate number of constituent cells at any stage may be calculated. In a previous paper (1939) the author has made an analysis of cell size in various regions of the cucurbit ovary—epidermis, outer wall, inner wall and placental region—during its development from early primordium to maturity, in twelve lines belonging to four genera. The purpose of the present study was to measure the volumes of these regions during early development and to determine the increase in cell number, and thus the comparative rate of cell division, for each.

In the earlier paper it was shown that the size of the epidermal cells remains relatively constant

throughout most of the growth period, but that in the other tissues cell size increases during development, and more in the inner regions of the ovary than in the outer ones. In early growth cell size increases much less rapidly than organ size, so that most of the ovary growth must be by cell multiplication. At a given point, characteristic for each region and race, division ceases and much more rapid cell enlargement begins, the size of cell and organ thereafter increasing equally. In all races but one (*Cucumis Anguria*) cell division persists in all regions until the ovary diameter reaches at least 10 mm. Accurate measurements can be made on ovaries as small as 2 mm. in diameter. The developmental period of this organ from 2 mm. to 10 mm., therefore, serves as a convenient one for a comparative study of increase in cell number. In the paper already cited (1939, table 1), cell diameters for each region in all twelve races are given for ovary diameters of 2 mm. and of 10 mm., and these figures have been used in the present study. It should be remembered that cells of only the ground parenchyma and epidermis are considered, but that these constitute the bulk of the young ovary.

METHODS.—Volumes of regions were calculated from diameter measurements of equatorial cross sec-

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tions (fig. 1). At the two ovary sizes, the average diameter was determined for the placental region and for the circle of vascular bundles in the wall. The region between placenta and bundles, designated as "inner wall" and "middle wall" in the previous paper, is here treated as one layer, since the limit between the two tissues is not sharp enough for accurate measurement. This region in the present paper

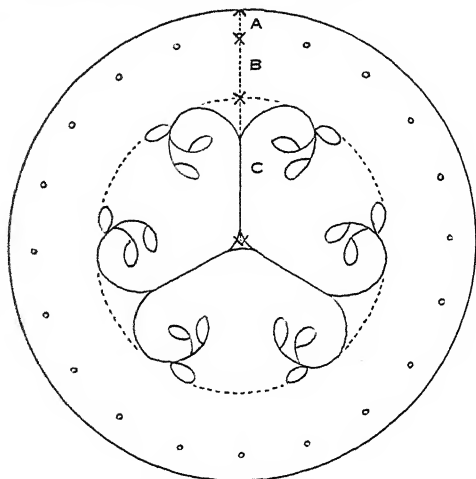


Fig. 1. Diagram of cross section of cucurbit ovary, showing the three inner regions measured. A, outer wall, between ring of large bundles and epidermis; B, inner wall, between placenta and bundle ring; C, placental region.

is distinguished as the "inner wall." The region between the vascular bundles and epidermis, as before, is termed the "outer wall." The average of the diameters for cells of the inner and middle wall in the previous paper was used as the cell diameter for the inner wall.

The absolute volume of the ovary and its various regions was impossible to determine from the data, but the cross sectional area of each at the equator could be measured with accuracy from these diameters. For purposes of comparison, cell number for each region at the two ovary diameters could be calculated from an equatorial slice, in thickness proportional to its diameter. The same regional volume relationships obtain, however, if the ovary and its regions are treated as concentric spheres; and since in all lines but one the ovary is approximately spherical, regional volumes and cell numbers have been calculated on this basis and thus approximate the actual values. The present study is concerned with comparative, rather than absolute, increases in cell number.

The volume of the placental region was determined directly from its diameter by the formula for spherical volume, $\frac{4}{3} \pi r^3$. Inner wall volume was found by subtracting this placental volume from the volume of a sphere the diameter of which was that of the bundle ring. This latter volume, in turn, was subtracted from the total ovary volume to obtain the

volume of the outer wall. Ovary surface was determined from its diameter, as $4 \pi r^2$.

Cell diameters were cubed to give approximate volume. Cell number for each region except the epidermis was determined by dividing this cell volume into the region volume. For the epidermis, cell number was found by dividing the surface area of the ovary by the surface area of the epidermal cells, found by squaring their diameters.

For comparative purposes the values reached by the methods here described are reasonably satisfactory, and the consistent relationships which they show give confidence in their essential accuracy.

From the ratio of cell number at 10 mm. to cell number at 2 mm., for each region, the number of cell generations can be computed by finding what power of 2 this quantity is. Thus if cell number at 10 mm. is 30 times that at 2 mm., there have been approximately 4.9 cell generations, since $30 = 2^{4.9}$. This value can readily be determined by dividing the logarithm of 30 (1.4771) by the logarithm of 2 (.301). That such a figure can be used as a measure of the number of cell generations depends on the assumption that every cell lineage is dividing at approximately the same rate. This seems probable, since the cells in a given region are similar in character and show only a limited range in size. It is not necessary to assume that division is rhythmic or simultaneous and there is no evidence that such is the case.

In Line G (*Cucumis Anguria*) where, for all regions except the epidermis, cell division ceases and vacuolation begins before the ovary reaches a diameter of 10 mm., the cell diameters at this ovary size obviously could not be used, and this line was omitted from the present study.

TABLE 1. Diameters in millimeters of placenta and vascular ring at the two ovary diameters and in the eleven lines.

Line	Diameter of placenta		Diameter of vascular ring	
	2-mm. ovary	10-mm. ovary	2-mm. ovary	10-mm. ovary
TA	1.04	5.84	1.68	9.04
SP	1.05	6.40	1.80	9.40
SRC	1.07	6.30	1.77	9.10
CF	1.12	6.60	1.84	9.50
M 35	1.20	6.67	1.83	9.36
MT	1.20	6.80	1.86	9.68
103	1.10	6.60	1.84	9.40
CN	1.30	7.96	1.70	9.36
GB	1.15	7.00	1.60	9.00
CL	1.20	6.90	1.68	9.10
WM	1.22	7.60	1.80	9.61

RESULTS.—In table 1 are given for the eleven lines the diameters of placenta and vascular ring at ovary diameters of 2 mm. and 10 mm. The first seven lines are of *Cucurbita Pepo*. Of these, TA, SP, and SRC are small fruited; CF, M35 and MT are large fruited, and 103 is intermediate. Lines CN, CL and GB are of *Lagenaria vulgaris*. CL is the "Hercules Club," much longer than wide, and GB is one of the

TABLE 2. *Regional volume (in cubic mm., or area in square mm. for epidermis), cell volume (in cubic micra, or area in square micra for epidermis), and cell number (in thousands of cells), in ovaries of 2 mm. and of 10 mm. in diameter, in the four regions of the eleven lines studied; together with the ratios of increase in region, cell, and cell number, and the number of elapsed cell generations during the growth of the large ovary from the small one.*

	2 mm.	10 mm.	Ratio of in- crease	No. of cell gen- erations
Line TA:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	64.00	68.89	1.08	
Cell number	196.	4,560.	23.23	4.54
Outer wall:				
Region vol.	1.706	136.78	80.2	
Cell vol.	2,000	6,332	3.17	
Cell number	853.	21,602.	25.33	4.66
Inner wall:				
Region vol.	1.894	282.53	149.0	
Cell vol.	2,571	17,576	6.82	
Cell number	737.	16,075.	21.82	4.44
Placenta:				
Region vol.	.589	104.29	177.0	
Cell vol.	8,000	64,000	8.00	
Cell number	74.	1,629.	22.13	4.46
Line SP:				
Epidermis:				
Ovary area	12.57	314.16	25.00	
Cell area	67.24	75.69	1.13	
Cell number	187.	4,151.	22.21	4.47
Outer wall:				
Region vol.	1.135	88.71	78.0	
Cell vol.	2,097	6,645	3.16	
Cell number	541.	13,349.	24.66	4.62
Inner wall:				
Region vol.	2.447	297.64	121.5	
Cell vol.	3,177	19,465	6.13	
Cell number	770.	15,291.	19.85	4.33
Placenta:				
Region vol.	.606	137.26	226.2	
Cell vol.	7,415	79,507	10.70	
Cell number	82.	1,726.	21.11	4.40
Line SRC:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	73.96	77.44	1.05	
Cell number	170.	4,057.	23.88	4.58
Outer wall:				
Region vol.	1.285	129.03	101.0	
Cell vol.	1,521	5,359	3.52	
Cell number	845.	24,077.	28.50	4.83
Inner wall:				
Region vol.	2.262	263.65	116.0	
Cell vol.	4,096	15,625	3.82	
Cell number	552.	16,873.	30.56	4.93
Placenta:				
Region vol.	.641	130.92	204.0	
Cell vol.	9,528	79,507	8.35	
Cell number	67.	1,647.	24.46	4.61

TABLE 2. *Continued.*

	2 mm.	10 mm.	Ratio of in- crease	No. of cell gen- erations
Line CF:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	132.25	125.44	.95	
Cell number	95.	2,504.	26.36	4.72
Outer wall:				
Region vol.	.927	74.68	80.6	
Cell vol.	2,863	8,000	2.79	
Cell number	324.	9,335.	28.82	4.85
Inner wall:				
Region vol.	2.526	298.39	118.0	
Cell vol.	4,173	16,975	4.07	
Cell number	605.	17,578.	29.04	4.86
Placenta:				
Region vol.	.736	150.53	204.5	
Cell vol.	10,078	74,088	7.35	
Cell number	73.	2,032.	27.83	4.80
Line M 35:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	68.89	64.00	.93	
Cell number	182.	4,908.	26.91	4.75
Outer wall:				
Region vol.	.980	94.23	96.3	
Cell vol.	1,405	4,492	3.20	
Cell number	698.	20,978.	30.07	4.90
Inner wall:				
Region vol.	2.304	273.99	119.0	
Cell vol.	1,728	7,189	4.16	
Cell number	1,332.	38,113.	28.61	4.84
Placenta:				
Region vol.	.905	155.37	172.0	
Cell vol.	8,000	54,872	6.86	
Cell number	113.	2,831.	25.04	4.64
Line MT:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	84.64	96.04	1.14	
Cell number	149.	3,271.	22.03	4.46
Outer wall:				
Region vol.	.819	48.15	58.7	
Cell vol.	2,248	5,545	2.46	
Cell number	364.	8,684.	23.83	4.57
Inner wall:				
Region vol.	2.465	310.29	126.0	
Cell vol.	3,443	17,576	5.11	
Cell number	716.	17,654.	24.66	4.62
Placenta:				
Region vol.	.905	164.64	182.0	
Cell vol.	15,625	117,649	7.53	
Cell number	58.	1,399.	24.17	4.60
Line 103:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	121.00	81.00	.67	
Cell number	104.	3,879.	37.33	5.23
Outer wall:				
Region vol.	.927	88.71	95.7	
Cell vol.	3,049	6,859	2.25	
Cell number	304.	12,933.	42.52	5.41

TABLE 2. *Continued.*

	2 mm.	10 mm.	Ratio of in- crease	No. of cell gen- erations
Inner wall:				
Region vol.	2,565	284.36	111.0	
Cell vol.	4,742	14,172	2.99	
Cell number	341.	20,065.	37.10	5.21
Placenta:				
Region vol.	.697	150.53	216.0	
Cell vol.	7,078	33,698	4.76	
Cell number	98.	4,467.	45.37	5.50
Line CN:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	84.64	100.00	1.18	
Cell number	148.	3,142.	21.16	4.40
Outer wall:				
Region vol.	1.616	94.24	58.3	
Cell vol.	1,728	4,492	2.60	
Cell number	935.	20,977.	22.43	4.49
Inner wall:				
Region vol.	1.422	165.28	116.2	
Cell vol.	3,049	15,625	5.12	
Cell number	467.	10,578.	22.65	4.50
Placenta:				
Region vol.	1.150	264.08	229.5	
Cell vol.	4,096	35,937	8.79	
Cell number	280.	7,348.	26.17	4.71
Line GB:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	187.69	161.29	.86	
Cell number	67.	1,948.	29.09	4.86
Outer wall:				
Region vol.	2.044	141.90	69.5	
Cell vol.	2,863	6,332	2.21	
Cell number	714.	22,409.	31.39	4.97
Inner wall:				
Region vol.	1.349	202.11	150.0	
Cell vol.	2,300	8,365	3.64	
Cell number	586.	24,161.	41.22	5.37
Placenta:				
Region vol.	.796	179.59	225.5	
Cell vol.	2,197	19,249	8.76	
Cell number	363.	9,330.	25.74	4.69
Line CL:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	125.44	171.61	1.37	
Cell number	100.	1,831.	18.27	4.19
Outer wall:				
Region vol.	1.706	129.03	75.7	
Cell vol.	2,986	15,625	5.23	
Cell number	571.	8,258.	14.46	3.85
Inner wall:				
Region vol.	1.578	222.56	141.5	
Cell vol.	3,724	32,768	8.79	
Cell number	424.	6,792.	16.03	4.00
Placenta:				
Region vol.	.905	172.01	190.0	
Cell vol.	6,029	74,088	12.30	
Cell number	150.	2,322.	15.47	3.95

TABLE 2. *Concluded.*

	2 mm.	10 mm.	Ratio of in- crease	No. of cell gen- erations
Line WM:				
Epidermis:				
Ovary area	12.57	314.16	25.0	
Cell area	106.09	148.84	1.41	
Cell number	118.	2,111.	17.82	4.16
Outer wall:				
Region vol.	1.135	58.90	51.9	
Cell vol.	2,406	6,332	2.63	
Cell number	472.	9,302.	19.72	4.30
Inner wall:				
Region vol.	2.103	234.85	111.8	
Cell vol.	3,375	20,346	6.03	
Cell number	623.	11,543.	18.53	4.21
Placenta:				
Region vol.	.951	229.85	242.0	
Cell vol.	6,859	85,184	12.43	
Cell number	139.	2,698.	19.47	4.28

constricted "bottle" races. *WM* is a watermelon (*Citrullus vulgaris*).

Table 2 presents the basic data of the paper. For each of the eleven races, and the two ovary diameters, there have been calculated the volumes in cubic millimeters of placental region, inner wall and outer wall; the average volume in cubic micra of the cells of each; the surface area of the epidermal cells in square micra; the cell number in each region; the ratio of the values for the larger ovary to those for the smaller; and the number of cell generations which would produce the increase in cell number.

Discussion.—A number of facts as to cell multiplication emerge from these data, notably that cell number increases at essentially the same rate in all regions, regardless of cell size; and that cell number and ovary surface tend to increase at the same rate. These facts have certain implications.

Cell size and division rate.—Even in the 2-mm. ovaries the cells of the inner regions are considerably larger than those of the outer ones, and these differences are accentuated during development by the more rapid increase in size of these inner cells. In such tissues each pair of daughter cells, before they divide again, evidently grow to a greater size than was reached by their mother cell. In the 10-mm. ovary as compared with the 2-mm. one, the cells of the outer wall are, on the average for eleven lines, about three times as large, those of the inner wall about five times as large, and those of the placental region between eight and nine times as large. Epidermal cell size shows little or no increase, or may even decrease slightly.

The ratio of increase in size of the various regions closely parallels that of their cells. These ratios differ markedly between regions and among the various lines, but the remarkable fact is that the relation of increase in regional volume to increase in cell volume (which evidently is the same as the ratio of increase in cell number, given in the table) tends to

be constant. Thus in Line *TA* ovary surface increases 25 times and epidermal cell area 1.08 times; outer wall volume 80.2 times and cell volume 3.17 times; inner wall volume 149 times and cell volume 6.82 times; and placental volume 177 times and cell volume 8 times. Despite these differences the ratio of region increase to cell increase is relatively uniform, being 23.23, 25.33, 21.82 and 22.13. The correlation between cell volume and region volume is close throughout. This must mean that increase in cell number, and thus rate of cell division, is much the same in the four regions studied. In the epidermis, cell number in the large ovary is, on the average for the eleven lines, 24.39 ± 1.57 times as great as in the small; in the outer wall, 26.52 ± 2.29 times as great; in the inner wall, 26.37 ± 1.94 times as great; and in the placental region, 25.18 ± 2.18 times as great. The average number of cell generations for these four regions is, therefore, $4.58 \pm .09$, $4.68 \pm .12$, $4.66 \pm .12$, and $4.60 \pm .12$. This close similarity in rate of cell division between big-celled regions and small-celled ones makes it clear that in this material, at least, increased cell size does not result in reduced division rate. In large cells the amount and rate of growth in each cell generation is evidently greater than in small cells, but whether this is in protoplasm or only in the vacuole is not known. Rate of cell division seems to be determined by some factor independent of cell size and operative throughout the entire organ.

It should be remembered that these figures are concerned entirely with the *comparative* rates of division and not with absolute rates. The four regions in a given ovary have obviously been growing for the same length of time, and increase in cell number in each can therefore be used as a fair measure of comparative division rate between regions. It cannot be used to compare absolute rates either as between regions or lines, since the length of the growth period between diameters of 2 mm. and 10 mm. is not given. As a matter of fact this is much the same in all lines.

That the relatively large and vacuolate cells of the innermost regions are still dividing is indicated by the fact that newly formed division walls can easily be recognized among them. In well-fixed material, mitotic figures are also evident. The author (and Bloch, 1941) has recently called attention to the frequent occurrence of divisions in comparatively large, vacuolate cells, and the tissues here considered are certainly not exceptional.

Cell number and ovary surface.—Not only is the rate of increase in cell number much the same between the various regions in a given line but it is very similar in the various lines. The average of all the ratios of increase is close to 25 times. This figure is also the ratio of increase of ovary surface, for as the diameter increases five times (from 2 to 10 mm.) the surface area of the ovary increases as the square of five. In other words, cell number throughout the ovary increases at approximately the same rate as does ovary surface.

Since increased surface makes possible increased gas exchange between ovary and atmosphere, there may be a physiological basis for this close relation between surface area and cell division. Not enough is yet known as to the relation between rate of respiration and the surface and volume of the fruit to make possible any definite conclusions in this regard.

This relation between cell number and ovary surface may have its origin in the unique behavior of the epidermis. All divisions in this tissue are anticlinal (perpendicular to the surface), and since cell size remains essentially constant here, division must be proceeding at just the rate which will maintain this cell size. As soon as two daughter cells reach this size they again divide, and epidermal cell number thus keeps direct pace with ovary surface. It may be that the constant cell size in the epidermis is a physiologically optimum one and that the rate of division necessary to maintain it acts as "pacemaker" for divisions in the inner and less closely regulated tissues.

The gradient in cell size.—These internal regions, which constitute the bulk of the ovary volume, must increase as the cube of the diameter, while the surface increases only as its square. Thus the volume of the 10-mm. ovary is 125 times as large as that of the 2-mm. one, while its surface is only 25 times as large. Since the cells of the inner tissues are increasing in *number* no faster than those of the epidermis (which keep pace with the surface and maintain a constant size), it is evident that their volume must be five times as great. This is true, in the aggregate, but the size increase is not distributed evenly, being considerably below five times in the outer wall and considerably above this in the placenta. This gradient may perhaps be related to the increased concentration of growth substance in the interior of the ovary which has been reported by Gustafson (1939), or to gradients in vitamin concentration, respiratory rate, pH, osmotic concentration, or other variables.

The similar gradient in cell size commonly observable in the primary parenchyma of stems and other massive organs may be due to the same causes—uniform rate of cell division throughout, constant epidermal cell size, and necessary increase in size of interior cells. How the variables of organ surface, cell division and cell enlargement are related is not clear, and is evidently an important problem in developmental physiology.

Progressive increase of cell size toward the center of the ovary and constant division rate result, as has been shown above, in a relatively greater increase in volume of the innermost regions and a relatively lesser increase of the outer ones. This also seems to occur commonly in massive parenchymatous organs. The writer has shown (1936) that in several stems, both of ferns and of higher plants, the pith is relatively larger and the cortex relatively smaller in later developmental stages than in earlier ones and in large stems than in small. Such differential growth between regions must evidently involve a regulation of plane of division in the more slowly growing regions. Since the placental region grows faster than

the inner wall, for example, the cells of the latter would be compressed were it not for the fact that there is among them an excess of radial divisions which increases the circumference of the tissue relative to its radius. The factor involved may be simply mechanical pressure, since it is well known that a cell tends to divide parallel to the direction of compression.

These changes in proportion with increasing size, in primary tissues, call to mind the suggestion of Bower (1930) that the changed surface-volume relationship in organs of different size are important factors in producing increased structural complexity. If, for example, constant cell size (as of tracheids or sieve tubes) is physiologically important in the interior of an organ which is enlarging, either in development or evolution, a greater frequency of cell division must take place within it than would normally occur; and if rate of division is proportional to surface, as the present study suggests, this increased division might be related to the increases in tissue surface, and to the resulting convolutions described by Bower in many enlarging structures.

Exceptional cases.—In a number of lines there are exceptions to the two general rules that in all regions cell multiplication occurs at the same rate and that increase in cell number is proportional to organ surface.

Conspicuous is the case of line *CL*, a race of "Hercules Club," narrowly cylindrical in shape. Its cell volumes at an ovary diameter of 10 mm. are considerably too large as compared with one of 2 mm., increasing (on the average of all regions except the epidermis) about 8.8 times as compared to the expected 5 times. The average increase in cell number is, therefore, only about 15 times instead of the usual 25 times. In this race, however (unlike the others studied), length grows considerably faster than width, so that at a diameter of 10 mm. the length of the ovary, and thus its actual volume, is about 5 times as great as it would be if all dimensions were growing at the same rate. At a diameter of 2 mm. ovary length is 10 mm., but at a diameter of 10 mm. its length is 250 mm. The volume of ovaries at these diameters is obviously much greater than spherical ones of the same diameter would be. Absolute volumes of the ovary and its three inner regions were determined, assuming them to be cylinders; and from these values, the volumes of regions and cells were determined, by extrapolation, for ovary volumes equivalent to those of spheres 2 mm. and 10 mm. in diameter. Volume relations are thus comparable with the other lines. Actual surface area, corrected for shape, and epidermal cell area, were similarly determined. This method assumes that rate of increase in cell and region is constant from small ovary sizes to the larger one here involved, and all facts indicate that this assumption is sound. After the corrections thus made for line *CL*, it was found that the increase in cell number in the placental region was 19.1 times, in the inner wall 20.7 times, in the outer wall 20.5

times, and in the epidermis 26.1 times, with generation numbers of 4.25, 4.37, 4.36, and 4.70, results agreeing much more closely with those in the other lines. Even with this correction, cell number increases somewhat less than expectation and cell size somewhat more. Evidently the radically changed form of the ovary has modified the usual developmental schedule.

Line *GB* has relatively more cell multiplication than the average, the increase in cell number being over 31 times. Ovary surface, because of the progressive constriction in ovary shape, increases more than would that of a sphere, and increased cell division is perhaps a result of this.

In line 103 the increase in cell number between the two ovary sizes in all regions is consistently high (averaging 40.6 times) and cell size is correspondingly low. This line is distinguished from the rest by being the only one with a "bush" instead of a "running" type of vine, in having been inbred much longer, and in possessing a very warty surface. The latter trait, which very considerably increases the surface of the ovary and fruit, may perhaps be related to the marked increase observed in cell number.

There are several specific differences between lines, however, which do not affect the amount of cell increase. Thus line *CF* has only about half as many cells at comparable stages as does line *M35* and they are much larger, but the ratio of increase in cell number is essentially the same in both. Lines *TA* and *MT* differ greatly in final size and in the relative proportions of their tissues, but this does not affect the amount of cell multiplication in each.

Ovary pattern and fruit size.—The gradient in cell size and thus the relative sizes of the three internal regions, are markedly different in ovaries which are to develop into small fruits and in those which will make large fruits. The former have a much smaller placental region and a much thicker outer wall than the latter. Thus in the three small-fruited lines *TA*, *SRC*, and *SP* the average radius of the placental region in the 2 mm. ovary, is .527 mm. and of the outer wall .125 mm., but in the three large fruited lines *CF*, *M35* and *MT* these values are .587 mm. and .092 mm. The vascular ring and the regions in which division persists longest (the walls) thus have their cells considerably nearer the surface in the ovaries which are to grow to greatest size. There may be some connection between these facts.

SUMMARY

In eleven lines of cucurbits, belonging to three genera, the increase in cell number between an ovary diameter of 2 mm. and one of 10 mm. was determined for placental region, inner wall and outer wall by measuring the volumes of these regions and dividing each by its average cell volume; and for the epidermis by dividing ovary surface by average epidermal cell area. Cell division was still taking place in all these regions.

Between these two ovary sizes, epidermal cell volume remains essentially constant but in the other regions cell volume increases, and at a higher rate in the inner than in the outer ones.

Regional volume also increases faster in the inner than in the outer portion of the ovary, and there is a close correlation between rate of increase in cell volume and in regional volume.

Cell number thus increases at essentially the same rate in all four regions, so that rate of cell division is independent of cell size or position.

The increase in cell number in all regions tends to be the same as the increase in ovary surface. Physiological implications of this fact are discussed.

The greater increase in volume of inner cells as compared to epidermis is a necessary result of the fact that the ovary volume increases faster than its surface but that rate of cell division is constant. Possible reasons for the gradient in cell size from surface to center are suggested.

The relation of the results of this study to various problems of growth and differentiation are discussed.

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DEVELOPMENT AND RELATIVE GROWTH IN OVARIES OF IRIS FULVA AND I. HEXAGONA VAR. GIGANTICAERULEA¹

Herbert Parkes Riley

STUDIES OF the morphological characters, geographical distribution and pollen fertility of a number of types of *Iris* in southeastern Louisiana (Viosca, 1935; Foster, 1937; Riley, 1938, 1939) have shown that *Iris fulva* Ker-Gawler, *I. hexagona* var. *giganticaerulea* (Small) R. C. Foster, *I. virginica* L. and *I. brevicaulis* Raf. are valid species, while *I. vinicolor* Small, *I. Thomasii* Small, *I. chrysophoenicia* Small and other species and varieties from that locality probably had a hybrid origin.² In studying these forms morphologically it was noticed that in *I. fulva* the ovaries and young capsules have six deep, wide grooves and six prominent, rather narrow ridges extending lengthwise, while in var. *giganticaerulea* the grooves are less deep and the ridges are wider and much less marked. In *fulva*, a cross section reveals a deeply indented outline while in *giganticaerulea* the indentations are much shallower and the outline appears scalloped. This difference is so marked that ovaries and young capsules of the two species can easily be distinguished. In spite of this noticeable difference during younger stages of development, the mature, fully grown capsules of both species are very nearly alike. The purpose of this in-

vestigation was to study how the fruit develops in both species and, by comparison, to determine, if possible, how inherent genetic differences may influence the pattern of development. The method of attack was to observe the shape of the ovary at the time it is first formed, to study the relative growth of the most important radii of the ovary throughout development and to determine what biological events are occurring during various stages of relative growth. The method of studying relative growth was suggested by Huxley (1932), who used it to compare the relative growth of an organ with that of the entire organism. Sinnott (1936) showed that this method is also applicable to dimensional relationships and not necessarily to a comparison of part with the whole. He pointed out that differences in shape of fruits of different species of gourd may be due to: (1) differences in shape which are visible as soon as the primordium of the ovary is formed; (2) constant differences in growth rate between length and width during development, even if the original sizes and shapes of the primordia are the same; and (3) differences in the final size of the fruit even though the original shapes and subsequent relative growth rates are the same in all species, provided that the value of *k* in Huxley's formula in the species studied is not 1.

MATERIALS AND METHODS.—The plants used for this investigation were wild plants growing in their native habitats in southeastern Louisiana. The study upon *I. fulva* is based upon a group of clones located about five miles north of Thibodaux and growing on the farthest slope of an alluvial ridge bordering a former deltaic stream. Collections were made at intervals of a week to ten days from early February until about the middle of August, 1938. The plants of

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² In one paper by the author (Riley, 1939) it was stated that Ponchatoula Blue was considered a hybrid between *giganticaerulea* and *virginica*; this statement was due to an error in transcription, since Ponchatoula Blue is probably a hybrid between *giganticaerulea* and *brevicaulis* or the result of a backcross of such a hybrid to *giganticaerulea*.

I. hexagona var. *giganticaerulea* were growing in a fresh water marsh on the eastern side of Bayou Barataria near its intersection with Bayou Villars. Dates of collection were approximately the same as those for *I. fulva*, since it was the author's usual practice to visit Bayou Barataria on Thursday of each week and follow each collection there with a trip to Thibodaux the following Saturday; during July and August, when the capsules had matured, collections were less frequent.

To determine the measurements of the radii, fresh ovaries and capsules were cut into several pieces, fixed in FAA or Craf, sectioned, and stained; the measurements were obtained from camera lucida drawings of the cross sections.

To study the relative growth of two radii, their measurements were plotted on logarithmic paper after which the points were inspected for the purpose of fitting the most accurate curve. In most cases the curve appeared to consist of two or more straight

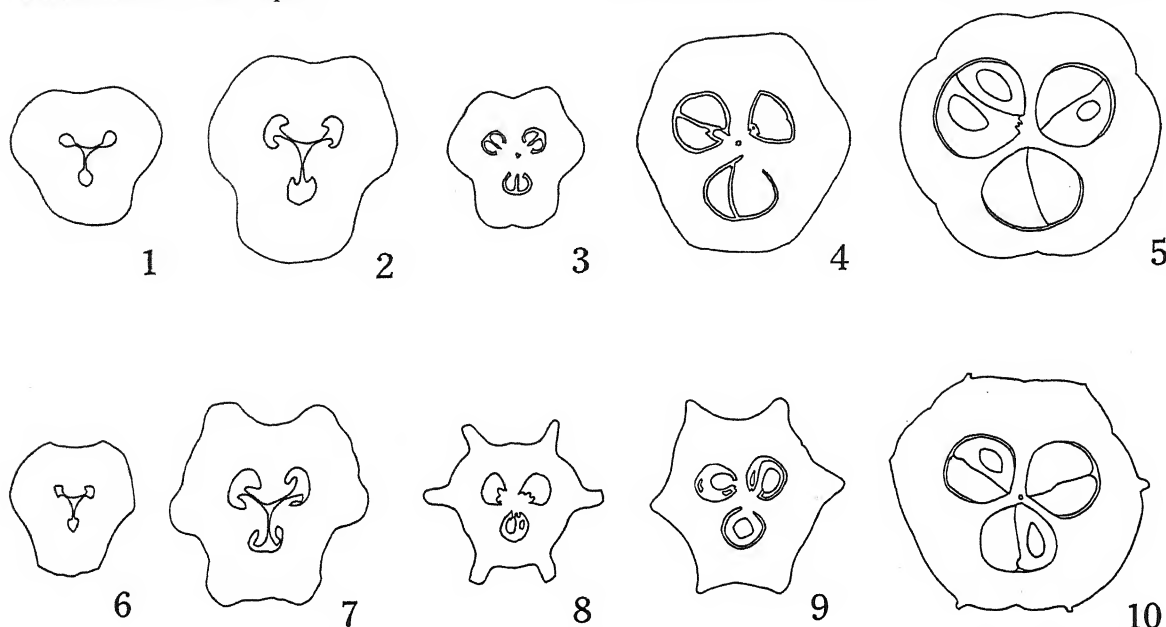
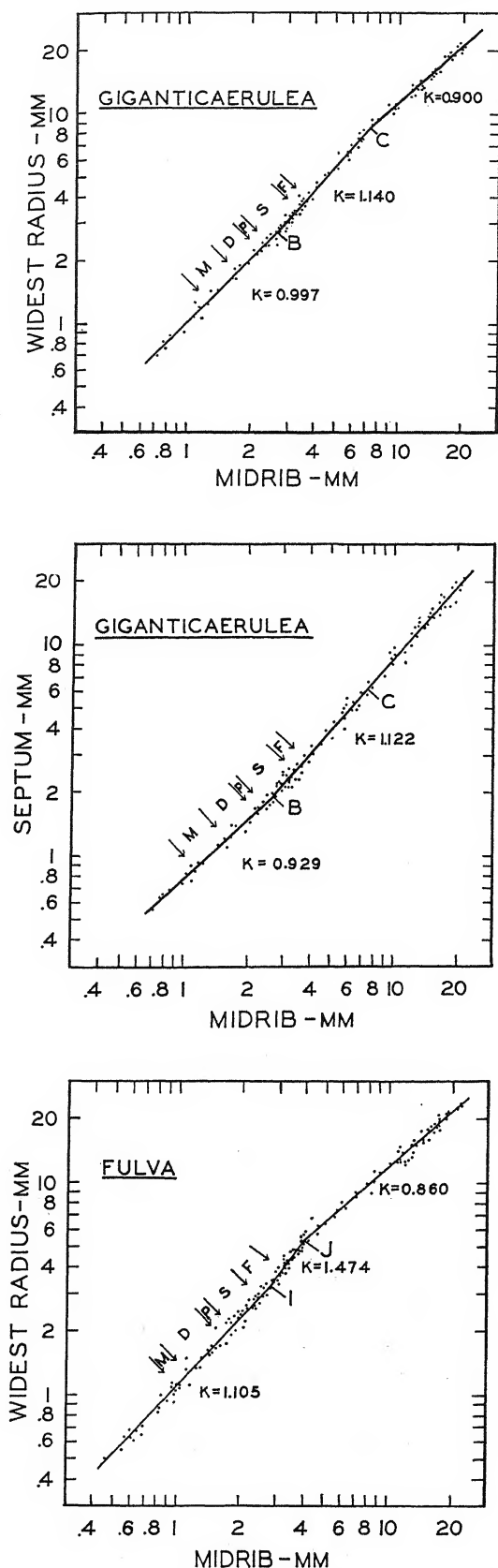


Fig. 1-10. Cross sections of *Iris* ovaries.—Fig. 1-5 are of *giganticaerulea* and figures 6-10 are of *fulva*.—Fig. 1 and 6. Cross sections of ovaries of young buds shortly after the organization of the ovary.—Fig. 2 and 7. Somewhat older buds showing the megasporangia growing into the locules.—Fig. 3 and 8. Flowers at the time of fertilization.—Fig. 4 and 9. Partially developed capsules.—Fig. 5 and 10. Mature capsules.—Fig. 1, 2, 6 and 7 are $\times 12$; fig. 3 and 8 are $\times 3$; fig. 4 and 9 are $\times 1.5$; and fig. 5 and 10 are $\times 0.75$.

To obtain a picture of the changes in development which determine the shapes of the ovaries of the two species, 145 ovaries and capsules of *fulva* of various sizes from less than 1 mm. to 40 mm. wide, and 114 ovaries and capsules of *giganticaerulea* between 1.2 and 40 mm. were studied. Cross sections at the widest part were used and measurements were recorded of the following radii for each ovary and capsule: (a) the midrib of the carpel, used herein as the radius of reference in studying relative growth, (b) the septum between two carpels, one of which included the measured midrib, and (c) the radius of greatest length, or "widest radius," which is usually found about half-way between the midrib and septum and, therefore, about 30° from the midrib. Indentations in the outline of the cross-section may be found at the septum and midrib. These represent the furrows. As the furrow at the septum is present from the beginning of ovary formation, it is referred to in this paper as the "primary furrow," while that at the midrib is referred to as the "secondary furrow."

lines with different slopes. When it was decided about how many such lines constituted the whole curve, the slope or value of k of each line was determined by the method of least squares. When these lines were plotted, they formed a continuous curve with one or more breaks at the intersections of the separate lines. The plotting of the curves was done with the advice and assistance of Professor Z. William Birnbaum of the Department of Mathematics of the University of Washington, whose kind assistance the author gratefully acknowledges. The least square values were calculated by Mr. Charles Alexander.

Since the number of points by which the slopes of the separate lines of the curves were determined was not infinite, these slopes are subject to errors of sampling, and their significance might therefore be questioned. While methods for the determination of the standard errors of k values have been devised, it was considered unnecessary to compute the standard errors in this case, for the interest here is in the general trend of the curves rather than in the exact values of k . It must be realized in considering these k



values, therefore, that they are of limited significance, but that they are sufficiently accurate to show the general trend of the curve.

To determine to what extent increase in ovary width is due to the relatively greater growth of the locule or of the wall, the ovary wall was plotted against the locule at all three radii. Also, the locule and ovary wall at the midrib were plotted against the locular region and ovary wall of both the septum and the widest radius. Again, in most cases the curve consisted of several intersecting straight lines. For comparison, corresponding intersections or breaks in the several curves are indicated by the same letter. Thus, the breaks in the curves of *giganticaerulea* are designated "B" and "C" while those in *fulva* are "H," "I" and "J."

Curves of relative growth have more significance if various places on the curve can be correlated with biological events. The development of the megaspore and megagametophyte was, therefore, studied from the slides from which the measurements had been obtained so that the stages of development would be known for ovules with midribs of various lengths.

OBSERVATIONS.—*I. hexagona* var. *giganticaerulea*.—Just after the ovary has been organized, the widest radius is the same length as the midrib and the septum is about 19 per cent shorter. Thus the ovary is circular in cross section except for the primary furrows at the septa of the three carpels (fig. 1). The midrib grows at about the same relative rate as the widest radius during the early development of the ovary, during meiosis, the development of the megagametophyte and until shortly after the polar nuclei have fused (fig. 11). During that time it grows relatively faster than the septum (fig. 12), so that a short time after the fusion of the polar nuclei (point B on both curves) the midrib and widest radius are still approximately the same length, while the septum is about 27 per cent shorter and the primary furrows are, consequently, considerably deeper (fig. 2). From that time until the capsule is partly mature, i.e., between 13 and 14 mm. in diameter (point C), the widest radius grows slightly faster than the midrib, and the septum grows faster than both. As the result of these new relative growth rates, the widest radius becomes longer than the midrib, and secondary furrows are thus produced at the midrib, while the septum becomes increasingly less short in relation to the midrib and the primary furrows become

Fig. 11-13.—Fig. 11 (above). Relative growth curve of the midrib and widest radius of *giganticaerulea*. A slight break in the curve occurs at B and a more pronounced break at C. The various stages in the development of the megagametophyte are indicated as follows: M = meiosis of megasporocyte; D = divisions of nuclei in embryo sac following meiosis; P = fusion of polar nuclei in embryo sac; S = seven-nucleate embryo sac before fertilization; F = fertilization.—Fig. 12 (center). Relative growth curve of midrib and septum of *giganticaerulea*. A break occurs at B. Symbols are the same as in figure 11.—Fig. 13 (below). Relative growth curve of midrib and widest radius of *fulva*. Breaks occur at points I and J. Symbols are the same as in figure 11.

less pronounced (fig. 3 and 4). The change in the slope of the line at point B is more marked in the curve of midrib and septum than in that of midrib and widest radius. In fact, as there is some scatter of points in the latter curve and as the shift in the value

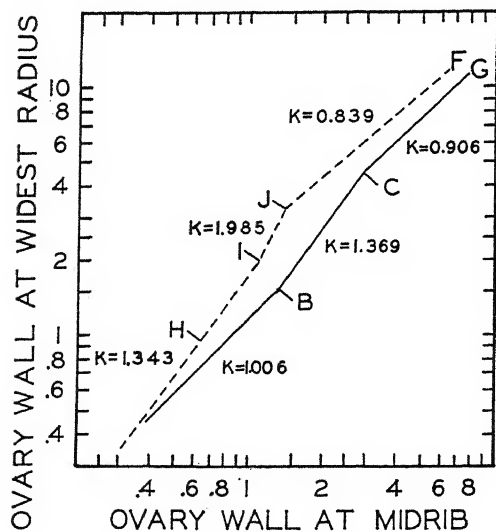
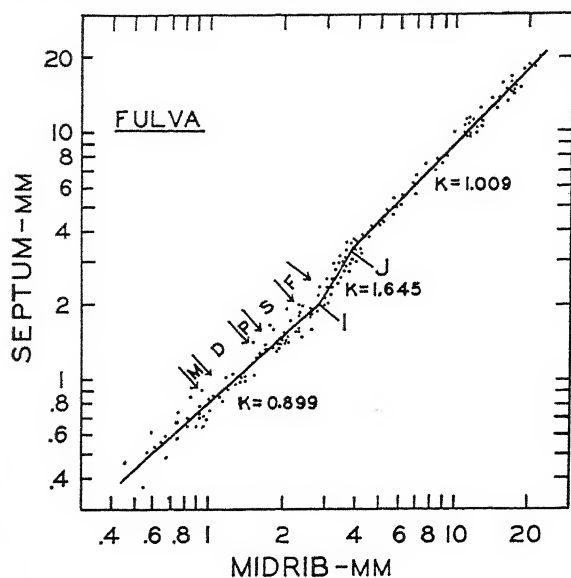


Fig. 14-15.—Fig. 14 (above). Relative growth curve of midrib and septum of *fulva*. Breaks occur at points I and J. Symbols are the same as in figure 11.—Fig. 15 (below). Relative growth curves of ovary wall at midrib and ovary wall at widest radius. Curve marked "G" is for *giganticaerulea* and that marked "F" is for *fulva*.

of k after B is slight, it might be almost equally justifiable to plot the curve as a straight line from its beginning to point C. That there is probably a slight change at B is indicated by the greater change at B in figure 15, the curve of ovary wall at the midrib and ovary wall at the widest radius. When the capsule is

about 14 mm. in diameter, the widest radius is about 16 per cent longer than the midrib and the septum is about 15 per cent shorter. During the remainder of capsule development, the midrib grows relatively somewhat faster than the widest radius but still relatively more slowly than the septum. When the capsule is mature, the widest radius is only about seven per cent longer than the midrib, and the septum is about three per cent smaller; the primary and secondary furrows are both rather shallow, and the capsule is almost circular in cross section (fig. 5).

The ovary is made up of three main parts: the placenta, the locular region, and the wall. Changes in relative growth rates of the radii may be due to changes in relative growth rates of one or more of these regions. The placental region is not so important a factor as the other two since it is relatively smaller, especially during later stages of development, and also because the relative growth rates of the placental region are the same for all radii. The locular region and wall, however, are much more important.

Prior to point B in figure 11, both the midrib and widest radius are growing at the same relative rate, because the wall at the midrib is growing at the same relative rate as the wall at the widest radius (fig. 15), while the locular regions at the two radii are also growing at the same relative rate (fig. 16). Increase in the diameter of the ovary is due more to the locular region than to the wall at this time, because the locular regions are growing relatively faster than the corresponding walls (fig. 17 and 18), although relatively at the same rate in both radii.

Between the first and second breaks in the curve (points B and C), the widest radius is growing slightly faster than the midrib because the wall at the widest radius is growing faster than the wall at the midrib. Actually, the slope of that part of the curve of the walls at the midrib and widest radius is steeper than the slope of the corresponding part of the curve of the two radii. This is to be expected, since the walls make up only a part of the radii, and the locular region and placenta at the widest radius are growing at the same relative rate as the locule and placenta at the midrib. During this part of relative growth, the locular regions at both radii are growing faster than their respective walls. Since the widest wall is growing faster than the midrib wall while the locular regions are growing at the same rate, the locular region at the widest radius should not be growing so rapidly in relation to its wall as the locule at the midrib is growing in relation to its wall. That this is true can be determined by examining the slopes of the curves between points B and C in figures 17 and 18.

After the second break in the curve (point C), the midrib grows slightly faster than the widest radius, because the wall at the midrib grows somewhat faster than the wall at the widest radius, while the locular regions of both radii are growing at the same relative rate. Again the locular region grows faster than the wall in each radius, and during the final stages of

capsule development growth in diameter of the capsule is due largely to growth of the locular region. Now, however, the midrib wall is growing faster than the wall at the widest radius, so the locular region at the widest radius should be growing faster in relation to its wall than the locule at the midrib is growing in relation to the wall at the midrib.

Figures 17 and 18 corroborate this. The relation of the parts of the radii to one another are summarized in table 1.

There is only one break in the curve of midrib and septum, and this occurs at the same time as the first break in the curve of midrib and widest radius (at point B). Before that break, the midrib grows faster than the septum because the wall at the midrib grows faster than the wall at the septum (fig. 19). The two locular regions grow at the same relative rate (fig. 20). The locular region grows relatively faster than the wall in both radii (fig. 17 and 21), showing that increase in thickness of the ovary is due largely to growth of the locular region of the ovary. Since the midrib wall grows relatively faster than the wall at the septum, the slope of the curve of the locular region and wall at the septum should be steeper than that of the curve of the locule and wall at the midrib. Figures 21 and 17 show that it is steeper.

After the break (point B) and for the remainder of ovary and capsule development, the septum grows faster than the midrib, because the wall at the septum grows faster than the midrib wall and the locular and placental regions are growing at the same relative rate. Again the locular region grows relatively faster than the ovary wall at both radii. Since, now, the wall at the septum is growing relatively faster than the wall at the midrib, the latter part of the curve of midrib locule and midrib wall is steeper than the corresponding part of the curve of septum locule and septum wall.

I. fulva.—The pattern of development in this species varies considerably from that of *giganticaerulea*. The shape of the ovary just after its formation is different, for the widest radii are between five and six per cent longer than the midrib instead of the

same length, and the septum is only about thirteen per cent shorter instead of nineteen as in the other species. Consequently, the ovary in cross section is more angular, there are shallow secondary furrows, and the primary furrows are less deep (fig. 6). From that time until just about fertilization (point I), the widest radius grows relatively somewhat more rap-

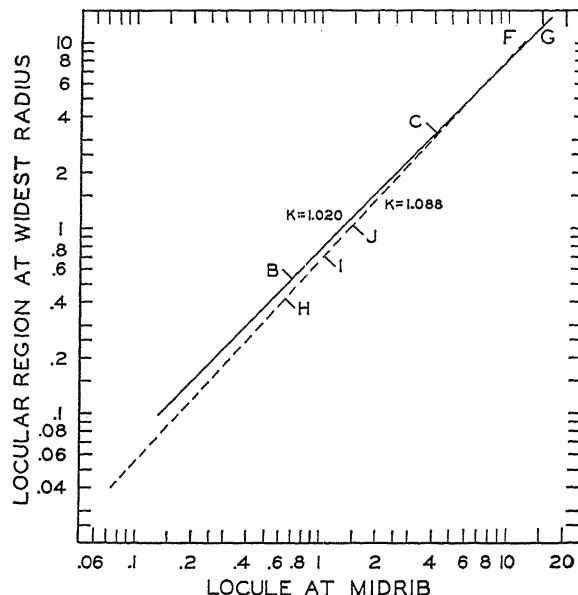


Fig. 16. Relative growth curves of locule at midrib and locular region at widest radius of *giganticaerulea* and *fulva*.

idly than the midrib (fig. 13), while the septum grows noticeably more slowly (fig. 14). At fertilization the widest radius has become 18 per cent longer and the septum 29 per cent shorter than the midrib (fig. 8). The primary furrows are, therefore, very deep and the six ridges are very marked. At this point, however, the widest radius and septum both grow very rapidly relative to the midrib, and these new relative growth rates obtain until shortly after

TABLE 1. Tabular summary of the relative growth rates of the midrib and widest radius, and midrib and septum, and of the walls and locular regions of *I. hexagona* var. *giganticaerulea*. M = midrib; W = widest radius; S = septum; M Wall = ovary wall at the midrib; W Wall = ovary wall at the widest radius; S Wall = ovary wall at the septum; M Loc = locule at the midrib; W Loc = locular region of the widest radius; S Loc = locular region of the septum. The figures in parentheses are the values of *k* as found in figures 11, 12, and 15 to 21.

	Before point B	Between points B and C	After Point C
Midrib and widest radius	M = W (0.997) M Wall = W Wall (1.006) M Loc = W Loc (1.020) M Loc > M Wall (1.305) W Loc > W Wall (1.314)	M < W (1.140) M Wall < W Wall (1.1369) M Loc = W Loc (1.020) M Loc > M Wall (2.223) W Loc > W Wall (1.731)	M > W (0.900) M Wall > W Wall (0.906) M Loc = W Loc (1.020) M Loc > M Wall (2.223) W Loc > W Wall (3.403)
Midrib and septum	M > S (0.929) M Wall > S Wall (0.799) M Loc = S Loc (1.004) M Loc > M Wall (1.305) S Loc > S Wall (1.781)	M < S (1.122) M Wall < S Wall (1.664) M Loc = S Loc (1.004) M Loc > M Wall (2.223) S Loc > S Wall (1.365)	M < S (1.122) M Wall < S Wall (1.664) M Loc = S Loc (1.004) M Loc > M Wall (2.223) S Loc > S Wall (1.365)

fertilization (point J). Thus, when the capsule is about 7.5 mm. in diameter, the widest radius is about 37 per cent longer than the midrib, while the septum is only about eight per cent smaller. The ridges are more pronounced, the secondary furrows have become deeper, and the primary furrows are less deep when compared to the secondary furrows at the midrib but appear very deep because the ridges

A study of the relative importance of ovary wall and locular region in determining the relative growth rates of the radii reveals a much more complicated situation than in *giganticaerulea*. The widest radius grows slightly faster relatively than the midrib until just before fertilization (fig. 13, point I), because the wall is growing faster (fig. 15) and the locular region slightly faster (fig. 16) at the widest radius

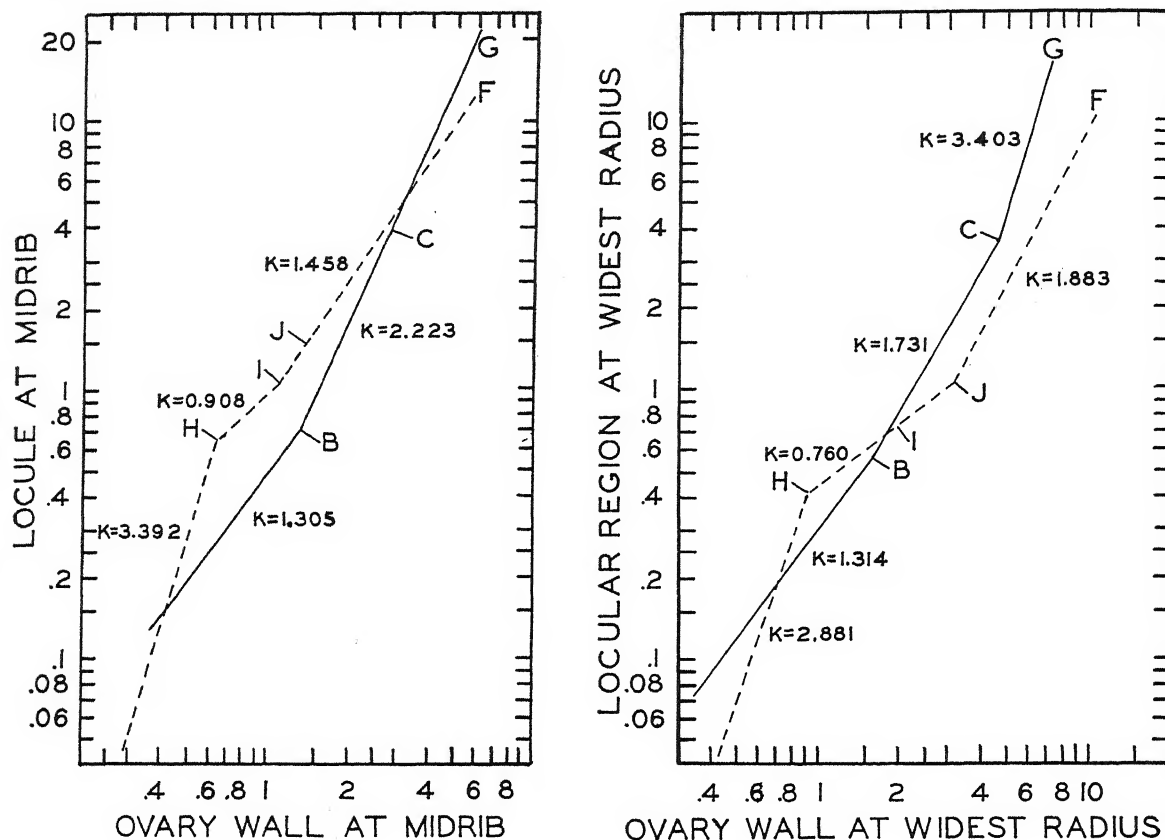


Fig. 17-18.—Fig. 17 (left). Relative growth curves of ovary wall at midrib, and locule at midrib, of *giganticaerulea* and *fulva*.—Fig. 18 (right). Relative growth curves of ovary wall and locular region at widest radius of *giganticaerulea* and *fulva*.

are so prominent on either side of them. From then until maturity, the midrib grows relatively somewhat faster than the widest radius, while the septum grows at about the same relative rate as the midrib. The slower relative growth of the widest radius when compared with the midrib makes the ridges and secondary furrows less prominent (fig. 9). As the septum is growing at almost the same relative rate as the midrib, it is growing somewhat faster than the widest radius, so the primary furrows are also becoming less deep, since the ridges on either side of them are becoming less prominent. At maturity (fig. 10), the capsule is almost circular, for the widest radius is only about nine per cent longer than the midrib and the septum is only about nine per cent shorter. The ridges have almost disappeared, and the furrows are mere indentations.

than at the midrib. Until just after meiosis when the megagametophyte has begun to develop (point H), the locular region grows relatively much more rapidly than the wall at both radii. Since the wall at the widest radius is growing somewhat faster than the midrib wall, the slope of this part of the curve of midrib locule vs. midrib wall is steeper than the corresponding part of the curve of locular region and wall at the widest radius, as figures 17 and 18 show. From point H until just at the beginning of fertilization (point I), the widest wall grows relatively faster than the locular region at the widest radius, and the midrib wall grows at the same relative rate as, or just slightly faster than the locule at the midrib. In spite of this great shift in relative growth rates of locular regions and walls of the same radii, the relation of the midrib as a whole to the entire widest radius is

unchanged because the wall and locule at the midrib retain the same relationship to the wall and locular region at the widest radius that they had previously. The change in relative growth rates within one radius is compensated for by the change within the other radius, so that the two radii remain relatively unchanged with respect to one another.

After fertilization has begun (point I) there is another change in some of the relative growth curves and the new values obtain until after fertilization (point J). During this period of relative growth, the locular region at the widest radius continues to grow just slightly faster relatively than the locule at the midrib (fig. 16), and the wall at the widest radius continues to grow faster relative to the locular region of the widest radius and at the same relative rate as between points H and I (fig. 18). The wall at the midrib, however, now grows at a considerably slower rate relative to both the locule at the midrib (fig. 17) and the wall at the widest radius (fig. 15). The entire widest radius, therefore, grows very much faster than the midrib during this period (fig. 13).

After point J and during, therefore, almost all capsule development, the wall at the widest radius slows up greatly relative to the midrib wall and actually grows a little more slowly than the wall at the midrib (fig. 15). It is because of this change in the relative growth rates of the walls that the midrib grows slightly faster than the widest radius during the maturity of the capsule. During this period the locular regions of both radii are growing relatively faster than their respective walls. The locule at the midrib grows relatively faster than the wall at the midrib (fig. 17), which in turn grows slightly faster relative to the wall at the widest radius. The locular region at the widest radius is growing slightly faster than the locule at the midrib. Therefore, the curve of the locular region and wall at the widest radius should be somewhat steeper than that of the locule and wall at the midrib. Figures 17 and 18 show that this is so.

Until just before fertilization (point I), the midrib grows somewhat faster in relation to the septum (fig. 14) because the wall at the midrib grows somewhat faster than the wall at the septum (fig. 19), while the locular regions at the two radii grow at the same relative rate (fig. 20). Until after meiosis (point H), the locule at the midrib grows relatively faster than the midrib wall, and the locular region of the septum grows relatively faster than the wall at the septum. Since the wall at the midrib is growing slightly faster than the wall at the septum, the curve of locular region and wall at the septum should be slightly steeper than that of the locule and wall at the midrib before point H. A comparison of figures 17 and 21 shows that this is so. Between points H and I, the locular region at the septum grows at the same relative rate as the wall at the septum, while the midrib wall now grows slightly faster than the locule. The shifts at point H in the relative growth rates of locular regions and walls of the same radii do not affect the relative growth rates of the entire

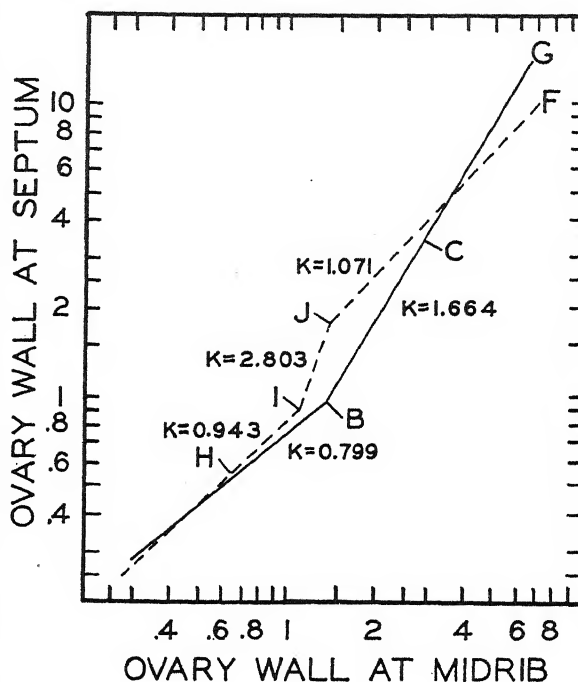


Fig. 19. Relative growth curves of ovary wall at midrib and ovary wall at septum of *giganticaerulea* and *fulva*.

radii, since the locular region and wall of one radius still grow at the same relative rates as the corresponding regions of the other radius. The change at point H is a very interesting one. Before that point, the locular regions of both radii grow considerably faster than the walls at the same radii, but since the locular region at the septum grows slightly faster in

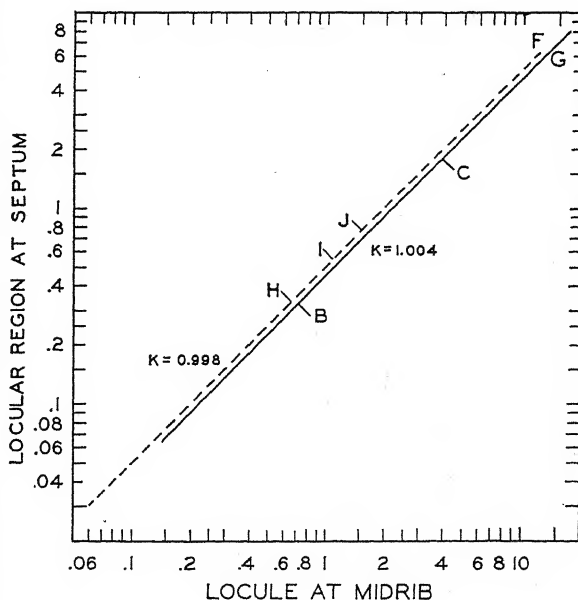


Fig. 20. Relative growth curves of locule at midrib and locular region at septum of *giganticaerulea* and *fulva*.

relation to its wall than the locule at the midrib does in relation to its wall, and since the locular regions grow at the same rate in relation to each other, the midrib wall grows slightly faster relative to the septum wall and, therefore, the midrib as a whole grows slightly faster than the septum as a whole. After point H, however, and until point I, the locular regions cease to grow faster relative to their walls. The locular region at the septum is now growing at the same rate as both the septum wall and the locule at

spect to relative growth is: septum wall, locular regions at septum and midrib, and midrib wall.

During most of capsule development (after point J), the locule at the midrib grows faster than the midrib wall, maintaining the same relative growth rate that was established at point I (fig. 17). The relative growth rates of the regions of the septum are now changed, however, for after point J the locular region grows faster than the wall (fig. 21). Since the midrib locule in relation to its wall grows somewhat faster than does the locular region at the septum in relation to the septum wall, the septum wall grows slightly faster than the midrib wall. In view of the fact that the two locular regions and placental regions are growing at the same rate, the somewhat faster growth of the septum wall relative to the midrib wall has but slight effect on the relative growth rate of the midrib and septum as a whole.

The relative growth rates in *fulva* are tabulated in table 2.

DISCUSSION.—The differences in form of the two species are due first to differences in initial shape and second to differences in relative growth rates. Initially, the ovary of *fulva* is slightly angular and has six small but definite ridges, while that of *giganticaerulea* is circular except for the three primary furrows. In the earlier stages of ovule development, the widest radius grows somewhat faster in *fulva* than the midrib but at the same relative rate as the midrib in *giganticaerulea*. During the same period the septum grows somewhat more slowly than the midrib in both species. Therefore, the ridges become increasingly more prominent in *fulva* but do not become prominent in the other species, while the primary furrows become deeper in both species but more so in *fulva*.

Coincident with fertilization, the widest radius and septum grow much faster in relation to the midrib in *fulva* than in *giganticaerulea*. These rates do not extend much past fertilization, but they increase greatly the ridged character of the *fulva* ovary during that period.

In the development of the capsule after fertilization, the midrib grows slightly faster than the widest radius in *fulva*, but in the other species it grows slightly more slowly until the capsule is partly formed and then slightly faster. In *fulva* the septum and midrib grow at almost the same rate during the entire development of the capsule, while the septum grows relatively faster in *giganticaerulea*. Thus, changes in growth rates continually diminish the difference between the two species which was so striking at about the time of fertilization.

It is interesting to note that in both species changes in the relative growth rates of two radii are due entirely to changes in the relative growth rates of their walls. In *giganticaerulea* the locular region at the widest radius grows continually at about the same relative rate as the locule at the midrib, while in *fulva* it grows just slightly faster throughout. In both species the locular region at the septum grows at the same relative rate as the locule at the midrib.

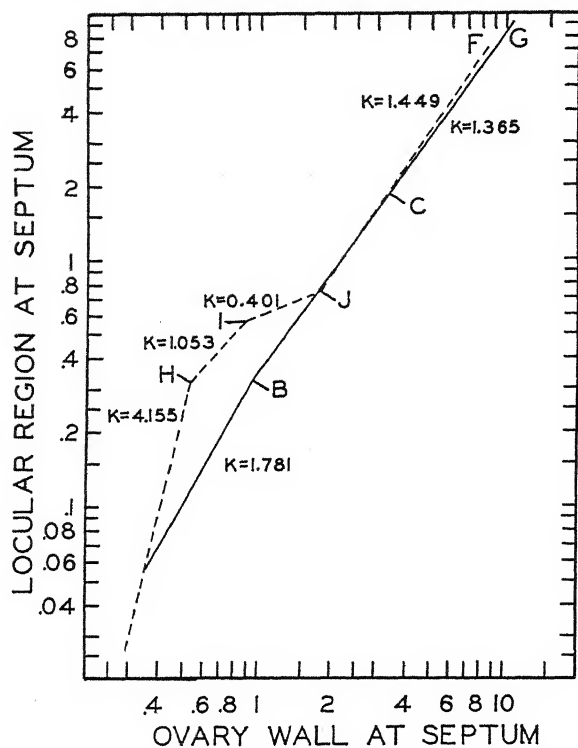


Fig. 21. Relative growth curves of ovary wall and locular region at the septum of *giganticaerulea* and *fulva*.

the midrib. Since, however, the wall at the midrib grows somewhat faster than the locule at the midrib and, therefore, than the locular region and wall at the septum, the same relation is maintained between the midrib wall and septum wall and between the midrib and septum that existed before point H. Between points I and J, the septum grows very rapidly in relation to the midrib (fig. 14) because the septum wall grows very fast in relation to the midrib wall (fig. 19), while the locular region at the septum grows at the same relative rate as the locule at the midrib (fig. 20). The wall at the septum grows much faster than the locular region (fig. 21). Since the locular regions at the midrib and septum grow at the same relative rate, while the wall at the septum grows faster than the locular region at the septum, the wall at the septum must grow faster than the locule at the midrib, which in turn grows faster than the midrib wall. During this period (from just before until shortly after fertilization), the order with re-

TABLE 2. Tabular summary of the relative growth rates of the midrib and widest radius, and midrib and septum, and of the walls and locular regions of *I. fulva*. The symbols are the same as for table 1. The figures in parentheses are the values of *k* as found in figures 13, 14, and 15 to 21.

	Before Point H	Between points H and I
Midrib and widest radius	M < W (1.105) M Wall < W Wall (1.343) M Loc < W Loc (1.088) M Loc > M Wall (3.392) W Loc > W Wall (2.881)	M < W (1.105) M Wall < W Wall (1.343) M Loc < W Loc (1.088) M Loc < M Wall (0.908) W Loc < W Wall (0.760)
Midrib and septum	M > S (0.899) M Wall > S Wall (0.943) M Loc = S Loc (0.998) M Loc > M Wall (3.392) S Loc > S Wall (4.155)	M > S (0.899) M Wall > S Wall (0.943) M Loc = S Loc (0.998) M Loc < M Wall (0.908) S Loc = S Wall (1.053)
	Between points I and J	After Point J
Midrib and widest radius	M < W (1.474) M Wall < W Wall (1.985) M Loc < W Loc (1.088) M Loc > M Wall (1.458) W Loc < W Wall (0.760)	M > W (0.860) M Wall > W Wall (0.839) M Loc < W Loc (1.088) M Loc > M Wall (1.458) W Loc > W Wall (1.883)
Midrib and septum	M < S (1.645) M Wall < S Wall (2.803) M Loc = S Loc (0.998) M Loc > M Wall (1.458) S Loc < S Wall (0.401)	M = S (1.009) M Wall < S Wall (1.071) M Loc = S Loc (0.998) M Loc > M Wall (1.458) S Loc > S Wall (1.449)

The relative growth rates of any two locular regions do not change from the beginning of ovule formation to the end of capsule development. Therefore, all changes must be due to changes in the relative growth rates of the ovary walls. The relative growth rates of the locular region and the wall of the same radius may change, but the locular region of one radius does not change in relation to the locular region of another radius.

In view of recent studies on hormones and fruit development, one is tempted to hypothesize a hormonal control of these relative growth rates. While such a suggestion seems very probable, the author has no experimental evidence to substantiate it.

SUMMARY

Various radii of cross sections of developing capsules of *Iris fulva* and *I. hexagona* var. *giganticaerulea* are compared. Although corresponding capsule dimensions of these two species are not very different

in the very early developmental stages and at maturity, they differ considerably in the intervening stages, since *fulva* has a much more ridged and furrowed ovary than *giganticaerulea*.

By means of logarithmic plots of one radius against another at different developmental stages, an analysis of the relative growth rates of the radii has been made. The linear relationships obtained are interpreted in the light of Huxley's heterogenic growth formula. The differences in the capsule development of the two species are described in terms of differences in the relative growth rates of their radii.

Similar logarithmic plots of the locular regions of two radii and of the ovary walls of the same radii show that differences in the relative growth rates of the radii are due chiefly to differences in the relative growth rates of the ovary walls at those radii.

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SOME RECENTLY COLLECTED SPECIMENS OF SCHIEDEA
(CARYOPHYLLACEAE) AND OF MEXICAN
COMPOSITAE ¹

Earl Edward Sherff

SCHIEDEA haleakalensis Degener & Sherff, sp. nov.—Frutex glaber, circ. 6 dm. altus; caulibus multis, subadsurgentibus et inter se laxè innexis, gracilibus, stramineis, fragilibus, ligneis (nodis tumidis, internodiis circ. 2 cm. longis); ramis foliosis, viridibus, saepe praecipue ad nodos subpurpurascenscentibus. Folia 4–8 cm. longa et 2–3 mm. lata, carnosa, pallida, subflaccida, 1-nervia, linearia, nitida, apice acuta, basi sessilia subamplexicaulique, submarcescentia, integra, marginibus incrassata, nervo mediano supra profunde sulcata infra prominenter lata crassaque. Flores numerosi, in paniculis erectis 3–5 cm. longis et 2 cm. latis dispositi; pedicellis gracilibus, puberulentis, circ. 4 mm. longis, basi 2-bracteolatis. Sepala 3 mm. longa, viridia, ovata, subobtusa, glabrata. Staminodia sepalis paulo breviora, subalba, linearia, apice obtusa, basim versus tumida subflavidaque. Stamina circ. 4 mm. longa, ea cum sepalis alternantia quam altera paulo breviora; filamentis gracilibus, albis; antheris pallido-flavis. Ovarium pallido-viride, glabrum, subglobosum; stylis 3. Capsula circ. 4 mm. longa. Semina compressa, orbicularia, 0.7 mm. lata, fusco-griseo-brunnea, colore hebetia, implicate profundeque stellato-sulculata.

Glabrous, about 6 dm. tall, shrub with many somewhat assurgent and loosely intertwined slender stramineous brittle woody stems having about 2 cm. long internodes and swollen nodes; leafy twigs green, often shaded with purple, especially at nodes. Leaves 4–8 cm. long and 2–3 mm. wide, fleshy, pale, somewhat flaccid, 1-nerved, linear, shiny, acute at apex, slightly clasping at sessile base, with midrib deeply sulcate above and prominently wide and thick below, entire with thickened margin, somewhat marcescent. Flowers numerous, on about 4 mm. long slender puberulent pedicels bibracteolate at base to form 3–5 cm. long and 2 cm. wide erect panicles. Calyx with 3 mm. long green ovate somewhat obtuse glabrate sepals. Staminodia slightly shorter than sepals, whitish, linear, with obtuse apex; swollen and yellowish near base. Stamens about 4 mm. long, those alternating with sepals slightly shorter than others; filaments slender, white; anthers pale yellow. Ovary pale green, glabrous, subspherical; styles 3. Capsule about 4 mm. long. Seeds compressed, orbicular, 0.7 mm. wide, dark grayish-brown, dull, intricately and deeply stellate-grooved.

Specimens examined: *Otto Degener, Emilio Ordoñez, & Felix C. Salucop* 12,695, Mt. Haleakala cliffs on west side of Kaupo Gap, eastern Maui, Hawaiian Islands, August 11, 1939 (type, N. Y. Bot. Gard.).

The type material, of which several specimens are being distributed to herbaria, was found growing

beyond the reach of wild goats on the arid cliffs that extend from Mt. Haleakala southeast along the western side of Kaupo Gap, on the Island of Maui. It is named, not for the great rift-valley known as Haleakala Crater, but for the mountain on which it grows. The species is related to *S. kealiae* Caum & Hosaka, of the dry cliffs about Kawaihapai, on the Island of Oahu.

BIDENS HETEROSPERMA A. Gray, Pl. Wright, 2: 90. 1853. In my monograph on the genus *Bidens* (Field Mus. Bot. Ser. 16: 381. 1937), this species was said to grow as far south as the Territory of Baja California and the states of Sonora and Chihuahua, Mexico. The range is now seen to be extended into the State of Sinaloa, Mexico, by the following two collections: *Howard Scott Gentry* 6,428, steep, moist canyon slope with mixed dominants, pine forest area, alt. $\pm 6,000$ –7,000 feet, Ocurahui, Sierra Surotato, September 1–10, 1941 (Field); *Gentry* 6,469, northern exposure, steep, moist canyon slope under mixed dominants, pine-oak forest, alt. $\pm 4,000$ –5,000 feet, Quebrada de Mansana, Sierra Surotato, September 10–14, 1941 (Field).

COREOPSIS CONGREGATA Blake, Jour. Wash. Acad. Sci. 19: 275. 1929.—Hitherto, this species has been known only from the type collection, obtained by the late Miss Ynes Mexia, no. 445. She had described it as common at altitude of 1,200 meters, growing in masses, in damp places in openings in oak and pine forests, trail from El Batel to Pica de Aguila, Sierra Madre, State of Sinaloa, Mexico, November 14, 1925. Recently additional material has been collected by Mr. Gentry, his no. 6,326, likewise in the State of Sinaloa. The label in the Herbarium of Field Museum bears the data: on moist boulders; rays orange with yellow tips; on steep, moist, shady canyon slope with mixed dominants; Ocurahui, Sierra Surotato, September 1–10, 1941.

DAHLIA Gentryi sp. nov.—Herba perennis, erecta, gracilis, pauciter ramosa, forsitan 5–10 dm. alta, radicibus non visis; caule plus minusve tereti, glabro, numerosè sulcato, infra circ. 2.5 supra ± 1.5 mm. crasso, internodiis 6–9 cm. longis. Folia opposita, graciliter petiolata petiolo sparsissime minutissimeque piloso usque ad 5.5 cm. longo, petiolo adjecto circ. 1.3–1.5 dm. longa, lamina bi- vel inferne tripinnata, rhachi gracillima ± 0.5 mm. lata, segmentis primariis 3 vel 5, gracillime petiolulatis petiolulo 1–3.5 cm. longo; ultimis saepe ternis, anguste ovatis vel rarius lanceolatis, 2–4.5 cm. longis et 0.8–2.4 cm. latis, apice plus minusve acuminatis basi late cuneatis vel oblique rotundatis, margine hispidulo-ciliato acriter 1–6-serratis pro utroque latere, membranaceis, faciebus secundum venulas moderate (et supra minutissime) alibi sparsissime adpresso-hispidulis, in-

¹ Received for publication January 20, 1942.

fra pallidioribus et eleganter reticulato-venulatis. Capitula perpauca (± 3), gracillime pedunculata pedunculo ± 1.3 dm. longo, radiata, ad anthesin ± 3.5 cm. lata et ± 1.2 cm. alta. Involucri bracteae exteriores circ. 5 vel 6, ad anthesin reflexae, subcarnosae, nunc oblongae vel lineari-oblongae nunc lanceolato-ovatae, glabratae, apice subacutae, 7–9 mm. longae et 2–3 mm. latae; interiores erectae, ovato-oblongae, apice rotundato-obtusae, ± 12 mm. longae et ± 5 mm. latae. Flores ligulati ± 8 , flavi; ligula obovata apice irregulariter 2- vel 3-dentata, ± 1 cm. longa. Flores disci plus minusve flavi. Ovaria plana, circumambitu orthogonio-oblonga vel -cuneata, apice calva, alibi glabra vel marginibus perpauciter setulosa. Achaenia non visa.

Perennial herb, erect, slender, sparingly branched, perhaps 5–10 dm. tall, roots not seen; stem more or less terete, glabrous, multisulcate, infernally about 2.5 mm., supernally ± 1.5 mm., thick, internodes 6–9 cm. long. Leaves opposite, slenderly petiolate (petiole very sparsely and very minutely pilose, up to 5.5 cm. long), about 1.3–1.5 dm. long including the petiole; blade bi- or infernally tripinnate, rhachis very slender and ± 0.5 mm. wide, the 3 or 5 primary segments very slenderly petiolulate with a petiolule 1–3.5 cm. long; ultimate segments often in threes, narrowly ovate or more rarely lanceolate, 2–4.5 cm. long and 0.8–2.4 cm. wide, at apex more or less acuminate, at base cuneate or obliquely rounded, at margin hispidulous-ciliate and with 1–6 serratures to each side, membranaceous, along the small veins of both faces moderately (and on upper face most minutely) elsewhere very sparsely appressed-hispidulous, infernally rather pallid and neatly reticulate-

venulate. Capitula very few (± 3), pedunculate (peduncle very slender and ± 1.3 dm. long), radiate, at anthesis ± 3.5 cm. wide and ± 1.2 cm. tall. External involucral bracts about 5 or 6, at anthesis reflexed, somewhat fleshy, now oblong or linear-oblong now lanceolate-ovate, glabrate, at apex subacute, 7–9 mm. long and 2–3 mm. wide; interior bracts erect, ovate-oblong, at apex rounded-obtuse, ± 12 mm. long and ± 5 mm. wide. Ligulate florets ± 8 , yellow; ligule obovate, at apex irregularly 2- or 3-dentate, ± 1 cm. long. Disc florets more or less yellow. Ovaries flat, in outline rectangular-oblong or -cuneate, at apex bald, elsewhere glabrous or at margins very sparingly setulose. Achenes not seen.

Specimens examined: *Gentry* 6,275, an openly branched perennial herb, on open lava pile, steep, moist, shady canyon slope with mixed dominants, in pine-forest area, alt. 6,000–7,000 feet, Ocurahui, Sierra Surotato, State of Sinaloa, Mexico, September 1–10, 1941 (type, Herb. Field Museum Nat. Hist.).

A species with ultimate leaf-segments somewhat similar to those of *Dahlia Merckii* Lehm. In that species, however, the leaves are merely once-pinnate, the peduncles mostly 2–4 times as long, the flowering heads much larger, the ligules lavender or rosaceous, etc. The type, together with the preceding and several other specimens collected by Mr. Gentry in Sinaloa, had been very kindly turned over to me by the Curator of the Herbarium at Field Museum of Natural History, Paul Carpenter Standley.

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THE FLORAL MORPHOLOGY OF THE CARYOPHYLLACEAE¹

Betty Flanders Thomson

THE CARYOPHYLLACEAE as a group include forms with small, simple flowers, such as *Sagina*, and others having large showy flowers such as *Dianthus* and *Lychnis*. Within the family a series can be arranged showing intergradations from a pentacyclic flower with many ovules in a completely septate ovary to a tricyclic flower with a simple perianth, one stamen whorl, and a unilocular, one-seeded ovary. This is usually, though not always, interpreted as a descending series, going in evolutionary sequence from the less reduced to the smaller forms. The present study was undertaken in order to analyze the vascular anatomy of representative flowers within this group and to interpret the bearing of data thus obtained on the relationships within the family and with other

groups. Such work should also throw light on the fundamental nature of the flower.

In the study of floral morphology emphasis was early laid on internal anatomy, especially the course of the vascular bundles, in a classic paper by van Tieghem in 1867. Further studies in this field were made by Henslow (1888 and 1890), Olbers (1895), Wester (1899), and Grélot (1897), who summarized the work on floral anatomy to that date.

The accumulated facts were interpreted specifically in terms of the anatomy of the leafy shoot by Eames and MacDaniels in 1925. This was further elaborated in detail by Eames in 1931.

Saunders has made a wide study of vascular patterns in flowers. Her work has been brought together into two volumes (1937 and 1939) which are dominated by the concept of carpel polymorphism but contain much detailed information.

These and other anatomical studies support the concept of the flower as a reduced axis beset with appendages which are in some manner homologous with foliage leaves and sporophylls. Other concepts, such

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as H. H. Thomas' derivation of the carpel from concrescent cupules, or J. M. Thompson's sporogenous axis, seem to disregard the structural likeness of floral parts to foliar organs, or to dismiss it as not highly significant.

Most workers agree that the primitive flower consists of many parts separately attached and spirally arranged upon a somewhat elongated receptacle; that cyclic arrangement and adhesion between cycles follows upon reduction in the number of members in a whorl and reduction in length of the axis or receptacle; that cohesion within a whorl is derived from the separate state. The vascular skeleton of plants is considered to be more resistant to change than other structures. It may or may not follow the fusions, reductions, or elaborations that the softer tissues undergo. For example, in the family Crassulaceae, tribe Kalanchoideae, the level of insertion of filament tissues upon the corolla varies from species to species, but the distance for which the vascular traces for petal and stamen are fused remains constant within the genus (Tillson, 1940).

LITERATURE ON THE CARYOPHYLLACEAE.—Several studies of floral ontogeny of the Caryophyllaceae have been published. The earliest is that of Payer (1857), who described the five whorls of floral parts as arising in acropetal sequence. Rohrbach (1868) found the same situation in *Silene*. Lister (1883) investigated carpel development in several species. She found that the early development of flowers with small incompletely septate ovaries is essentially the same as that of those having larger capsules with complete septa; that differences in form of mature capsules are due to differences in growth rates of ovary wall, septa, and central column. Schaefer (1890) studied other species with special attention to the rôle of the axis in ovary construction. Middleton (1914) followed the organogeny of *Saponaria officinalis*.

The vascular anatomy of various species of the Caryophyllaceae has been described by van Tieghem (1867), Henslow (1888 and 1890), Olbers (1895), Wester (1899), and Saunders (1925, 1932, and 1937). These accounts do not take into consideration the significance of the anatomy for relationships nor for floral morphology in general.

Pratt (1932) studied the floral anatomy of *Silene maritima* and concluded that incomplete development of stamens and petals is not due to anatomical defects of the vascular bundles. Dickson (1936) in a study of the Primulaceae described the vascular plans of *Melandrium album* and *Sagina procumbens*, and Dawson (1936) that of *Silene noctiflora* for purposes of comparison with the Polemoniaceae.

The present concept of the Centrospermae in the sense of Pax and Hoffmann (1934) as a natural line dates from a paper by Bentham in 1862. Bentham and Hooker (1862) listed the family among the Thalamiflorae, which starts with the Ranales. They did not specify phylogenetic relationships, but the sequence of genera implies that the family is a line of reduction. Wernham (1911) placed the Caryophylla-

ceae close to the Ranales and considered them the point of departure for the Primulaceae as well as for the more reduced Centrospermae families. Bessey (1915) arranged these families in a similar fashion, basing his ideas definitely on the strobiloid nature of the flower. Hutchinson (1926) regarded the Caryophyllaceae as derived from the Ranales through the Saxifragales and as the base from which arose many herbaceous lines, such as Centrospermae, Geraniales, and Primulales.

Eichler (1875) considered that the Centrospermae are a line of elaboration from the Polygonaceae and thought that the origin of the Caryophyllaceae is to be found among the Phytolaccaceae. The complete flowered Caryophyllaceae arose from a form like *Phytolacca* by conversion of the outer stamen whorl to petals and of the outer carpel whorl to stamens; from this the family shows a line of reduction. The same origin was proposed by Pax (1893 and 1927); by Bitzek (1928), who regarded the family as showing elaboration from a simple flower; by Lüders (1907), who saw reduction by abortion of a stamen whorl in three-whorled, and elaboration by dedoublement of the same stamen whorl in five-whorled members; and by Rendle (1938), who presented essentially the same views as Eichler.

Möbius (Warming, 1929) considers the Alsineae to be primitive within the family, with Paronychieae a series of reduction thence and Silenoideae elaborated with respect to insect pollination. Troll (1928) considers the family to be a line of reduction.

Coulter and Chamberlain (1903) regarded the Caryophyllaceae as the highest family of the Centrospermae. This entire line seemed to them unrelated to the Ranales and lines stemming thence. Pax and Hoffman (1934) consider that the Chenopod-Amaranth group gave rise to Phytolaccaceae and Caryophyllaceae as parallel lines. They consider the genera of the latter to show a sequence of elaboration from simple to large and complete flowers. Wettstein (1935) also upholds this point of view.

Much discussion has centered about the break in alternation sequence of stamen and carpel whorls in the Caryophyllaceae. Čelakovský (1875) discussed the various theories presented up to that time to explain this irregularity; he concluded that obdiplostemony in the androecium is secondary in this family and that so far as the gynoecium is concerned no single explanation fits all the cases. Schumann (1889) regarded the stamens in this family as really diplostemonous; contact during formation seemed to him to be the factor which determines the position of carpels. Stroebel (1925) reported that successive floral whorls are formed in acropetal sequence; that obdiplostemony arises by an increase in the calyx sectors, either by simple enlargement of primordia at an early stage, or by that in combination with a higher level of insertion of anteseptalous stamens; and that petal sectors show a corresponding tendency to reduction. Saunders (1935) postulates that in isomerous six-whorled flowers ($G=5 \text{ \& } 5$) in which midrib traces for all parts arise directly from the

stele, a "state of congestion" is set up after the formation of the second stamen whorl. Consequently the "outer" carpels (loculi) stand on petal radii unless some structural feature allows more space for the developing carpels; such features are a gynophore or the fusion of the basal parts of stamens with their subtending perianth parts.

Glück (1919) studied the morphology of floral leaves and concluded that sepals and petals are derived from foliage leaves, and stamens from petals. Homologues of the stipules of vegetative leaves can be seen in thin or membranous margins of sepals, in lateral wings on the petals of *Lychnis*, in glands at the bases of stamens as in the tribe Alsineae. Mattfeld (1938) saw in this view a solution of the problem of obdiplostemony in that if glands are stipules of antepetalous stamens, then petals are stipules of antepetalous stamens, which bear no such abaxial glands, and there are thus only two whorls between carpels and sepals. Arber (1939) showed by anatomical investigation that coronal teeth of *Lychnis* are simply dorsal invaginations and not ligules or enations or sutural ridges; that the lateral wings may be stipules as well as not; that Mattfeld's interpretation of petals as stipules is unsound. She pointed out that in the Caryophyllaceae petal plus stamen may "count" as one whorl or as two in the alternation sequence which ends with the formation of carpels.

The well-known variability in petal and stamen whorls of *Stellaria media* has been carefully studied by two investigators. Kraft (1917) reported that the number of these members can be controlled experimentally by varying the external conditions. Reduced flowers obtained thus reproduced the floral diagrams of typically oligomeric species. Reduction by abortion of members was observed in the floral ontogeny of some species. He concluded that simpler members of the family are reduced; that the most primitive forms in the family are those in the tribe Alsineae with complete flowers, the Sileneae showing such entomophilous adaptations as dichogamy and sexual dimorphism. Matzke has also studied variation in *S. media*, with special reference to symmetry. He found that extra or missing members are fairly well fixed in their location with reference to sepals (1929); that nutrition as influenced by variegation and by removal of flowers after anthesis plays an important part in determining the number of stamens formed (1930); that most variations which occur result in bilateral or radial symmetry of the individual flower (1932).

Vivian (1940) studied the structure and symmetry of flowers of *Sagina procumbens*. He found considerable regularity in the sequence of disappearance of the stamens.

MATERIALS AND METHODS.—Flowers in anthesis were preserved in a formalin-acetic acid-alcohol mixture, embedded in paraffin, sectioned serially at ten to fifteen microns, and stained in safranin and fast green. Complete series were studied, both transverse and longitudinal. Flowers were also cleared in 70 per cent lactic acid and studied whole. Some mate-

rial of buds and young flowers was presented to the writer already embedded in paraffin by Professor T. E. Hazen.

The vascular traces of flowers vary from substantial strands similar in size and composition to those of foliage leaves, as found in sepals, petals, and carpel walls, to procambial strands detectable only as elongated cells with small diameter and densely staining contents, such as those comprising the bases of stamen traces. Stamen traces are often faint and indefinite where they leave the stele, becoming stronger and showing mature xylem elements above.

In this paper the term *median lateral* carpellary traces refers to those which stand in the carpel wall at the ends of the septa. By *stelar internode* is meant a longitudinal segment of stele between the levels of departure of successive whorls of traces, uncomplicated by gaps or departing traces. The term *gynophore* is applied only to a stipe upon which the ovary is elevated. The term *perigyny* is used in quotation marks because the condition in this family clearly arises by the fusion of superposed whorls, and not by an up-growth of the torus.

The genus and species names used are those given by Gray's Manual (1908), or in the case of cultivated plants by Bailey (1938).

GENERAL MORPHOLOGY.—The flower of the Caryophyllaceae is typically hypogynous, actinomorphic, pentacyclic, and pentamerous or occasionally tetramerous.

The calyx is well developed, with sepals free in the tribe Alsineae, united into a tube in the Sileneae. In many members of the last tribe the lateral veins of adjacent sepals arise fused in pairs and stand on the commissure line; they are, therefore, often termed "commissurals." The commissurals may split into component parts near the base of the calyx, just below the sinuses, or not at all. Branches from the commissurals may or may not anastomose with those from the midrib.

Petals in the Sileneae are often differentiated into claw and limb, and often bear a corona or paracorolla at the juncture of these. In the other tribe the petals may be cleft or entire, isomerous with the sepals, or fewer in number, or altogether lacking.

Stamens in the Sileneae regularly form two complete whorls, of which the antepetalous set is pushed out against the petals in their upper reaches because of spatial relations. In the other tribe the stamen number may be reduced, either by disappearance of the complete antepetalous whorl or by abortion of part of a whorl, or both. The filament bases are frequently united into a very short tube and frequently adhere to the petal bases even above the level of separation of petals from each other.

Nectar tissue often forms a ring on the adaxial face of the tube formed by the united filament bases. In the Sileneae the glandular tissue is limited to this region. In the Alsineae this ring is somewhat thinner in the radial dimension, but glandular tissue may also form a collar around the base of each antepetalous stamen, extending below the level at which

the filaments become free, and often project in a lump on the abaxial side.

The syncarpous pistil is composed of five to two carpels with axile placentation, frequently, but as Goebel (1933, p. 1912) points out, falsely described as "free-central." Flowers up to the embryo sac stage of most species examined show at least the remains of complete septa. In later stages the septa, especially in the center and upper regions of the ovary, become delicate and fragmentary and may disappear entirely. Near the top of the ovary the partitions do not meet in the center, as though each carpel had a split or a hole in its adaxial face near the top. Septation is complete again below the bases of the separate styles. Ovules are attached to the placenta in double rows opposite the dorsal veins.

In the Alsineae species occur which show a reduction in the number of ovules and in the length of the placental column. In some of these the septa extend only part way up into the ovary cavity, with the result that the ovary is unilocular above. However, the placentation is not truly free-central even in these cases (Lister, 1883).

TRIBE SILENEAE.—*Dianthus* K5, C5, A5 & 5, G2.
—Four species were studied: *D. barbatus*, *D. chinensis*, *D. plumarius*, and *D. armeria*.

The pedicel is traversed by a complete vascular cylinder.

At the base of the flower a whorl of ten traces departs from the central cylinder. Of these, five small, simple strands become sepal midribs; each of the alternate set of large traces splits periclinally near the base; the resulting abaxial strand, a calyx commissural, immediately branches freely and enters the calyx tube; the adaxial strand becomes a petal midrib (fig. 3A-D). In *D. plumarius* petal traces arise from the same ribs on the stele as calyx commissurals, but the two are not really fused—that is, serial cross sections from the base upward show that the ribs from which the calyx commissurals depart are not completely retracted into the stelar cylinder until above the departure of the stamen traces (cf. fig. 18). In *D. armeria* the sepal midrib whorl is slightly but definitely lower in origin than the commissural (fig. 4); in the other species all ten traces depart as a single whorl. These traces are practically unbranched above the base and do not anastomose. There is a stelar internode above the departure of perianth traces.

Stamen traces arise independently from the stele. In *D. barbatus*, *D. chinensis*, and *D. plumarius*, the antesepalous whorl arises slightly below the antepetalous; in *D. armeria* both arise at the same level. In all species the antesepalous stand slightly farther out in the receptacle than the others (fig. 3F). Another stelar internode follows the departure of stamen traces.

Two dorsal carpellary strands arise at the base of the gynophore in *D. barbatus* (fig. 3F-K) and *D. chinensis*, just a short distance below the median laterals in *D. armeria* and *D. plumarius*. In *D. barba-*

tus, *D. plumarius*, and *D. chinensis*, forms with large capsules, an extra set of traces for the ovary wall arises between dorsals and true median laterals (fig. 3G). These extra strands die out at the top of the ovary. Dorsal traces enter the styles.

The true median laterals arise just below the loculi and traverse the ovary wall, one at each end of the septum. These traces fork at the base of the styles, and one branch from each lateral enters each style (fig. 3L-O), except in the case of *D. armeria*, where the laterals die out just after forking.

The remaining central vascular cylinder is traversed by a large central core of xylem (fig. 1-4). Higher up in the placenta this core becomes smaller and forms a band at right angles to the plane of the septum; in some sections it appears split along its long axis. It dies out at the topmost ovule traces. The pith disappears between a point just above the insertion of stamen traces and the level where median laterals depart.

Above the attachment of the ovules the septa withdraw from the center, so that a small part of the top of the ovary is incompletely septate. In *D. armeria* and *D. barbatus* the free ends of the septa are bilobed (fig. 3M). This, with the forking of the median laterals, suggests that the two carpels are incompletely fused at the top.

Henslow (1890) stated that in *D. barbatus* the petal traces arise directly from the stele, and each soon gives rise to a stamen trace. The observations of Olbers (1895) agree with those described in the present paper; she described also *D. deltoidea*, in which traces for both perianth whorls arise as a single complete ring which segments into its component parts. Wester (1899) gives figures of the ovary of this species showing the median laterals double at the top but apparently not entering the styles.

Gypsophila K 5, C 5, A 5 & 5, G 2.—Three species were studied: *G. muralis*, *G. elegans*, and an unidentified species, possibly *G. paniculata*, received already in paraffin.

The vascular tissue in the pedicel forms a complete cylinder.

At the base of the flower a whorl of ten traces departs. Of these strands, one set of five become sepal midribs which branch to varying extents. Each member of the alternate set of five immediately splits anticlinally into three strands, of which the median and by far the largest becomes a petal trace, and the others extend into adjacent sepals as lateral veins (fig. 5). In *G. muralis*, a very small flower, sepal laterals separate from only two or three of the petal traces, although all sepal laterals which extend to the base of the calyx arise thus. In *G. elegans* and *G.* species sepal laterals separate from all petal traces. In this genus the sepal margins are fused, but the vascular systems of the calyx segments are separate and independent except at their origin. In the fresh flower each segment consists of a central green strip bearing a network of veins, and a colorless margin.

Above the gaps from the perianth traces a single whorl of ten stamen traces arises independently from the stele (fig. 5, 7).

Above the stamen traces a pair of dorsal carpellaries arises. A pair of median lateral carpellary traces arises just below the bases of the ovary loculi; these consist of a single small bundle at each end of the septum. The ovary wall is supplied also by minor branches from these four strands and from the central remnant. The dorsal bundles extend into the styles; the median laterals die out at the base of the styles, forking dichotomously just before they do so in the case of *Gypsophila* sp. and *G. elegans*.

The tissue between the stamen bases and bottom of carpel loculi may be elongated into a gynophore as in *G. muralis* (fig. 7), or not, as in *G. elegans* (fig. 5), where the dorsal carpellaries leave the stele below the level of the glandular ring at the stamen bases.

After the carpel walls have been supplied, the central vascular remnant is a complete hollow cylinder which soon domes over and continues as a central mass of ovule traces. In *Gypsophila* sp. and *G. muralis* this placental mass has a central core of a few xylem elements. The downward connection of these is obscure, but seems to be to the inner edge of the vascular cylinder before the pith disappears. The placental septum separates near the topmost ovules into two lobes, as in the case of *Dianthus*. The placental vascular strand divides as the septa draw apart (fig. 6).

Saponaria K 5, C 5, A 5 & 5, G 2.—Two species were studied: *S. calabrica* and an unidentified species received in paraffin.

The stele in the pedicel is a complete cylinder.

The calyx traces depart as a whorl of ten, of which those on the midrib radii become free slightly below the others. In *S. calabrica* these whorls are markedly reflexed at their origin, following the form of the saccate base of the calyx (fig. 8).

Just above the calyx traces and slightly fused with them at the base arises another whorl of ten traces. The five of these standing on sepal radii are simple stamen traces; the alternate five soon split periclinally, each outer branch becoming a petal midrib, each inner a stamen trace.

The stele closes above these traces and the central pith soon domes over. Dorsal carpellary strands separate from the stele at the base of a gynophore in *S. calabrica*, just below the loculi in *Saponaria* sp. Both species have a single pair of unbranched median laterals, one at each end of the septum. These seem to fork at the top of the loculi before they die out, but the material at hand is young and the exact limits of traces are hard to determine. The placental vascular supply is single and central in the lower region, and splits into two at the top in *S. calabrica*.

In *Saponaria* sp. the entire placental column is ovuliferous; *S. calabrica* bears only two tiers of ovules about halfway up the loculi. The ovary is completely septate in both species, at least in the relatively young material available.

In *S. officinalis*, as described by Olbers (1895), the calyx traces are reflexed at the base and the stamen traces arise in two successive whorls, the antesealous lower. Saunders (1932) mentions the splitting of the placental strand above the level of the ovules in this species. Middleton (1914) stated that the ovary wall is lined with an epidermis which forms a definite absciss layer and thus actually cuts off the septa from the ovary wall.

Lychnis K 5, C 5, A 5 & 5, G 5.—Six species were studied: *L. alba*, *L. dioica*, *L. chalcadonica*, *L. flos-jovis*, *L. coeli-rosa*, and *L. coronaria*.

The vascular supply in the pedicel varies, being a practically complete cylinder in *L. flos-jovis* and *L. coeli-rosa*, a ring of five strands in *L. chalcadonica*, and a ring of eight or ten strands in *L. alba* and *L. dioica*.

The calyx is supplied by a whorl of ten traces. In *L. chalcadonica*, *L. coeli-rosa* (fig. 9), and *L. coronaria* (fig. 10) the traces on the sepal midrib radii arise slightly but definitely lower than the commissurals. In all species the midrib strands are relatively simple, but in *L. alba* ♀, *L. dioica* ♀, and *L. coronaria* the commissurals are larger and branch extensively near their origin. In *L. coeli-rosa* and *L. chalcadonica* the sepal traces are strongly reflexed in the base of the flower.

Petal traces arise in close conjunction with the calyx commissurals. They may arise from the same stelar rib as the commissurals, as in most species, or be slightly fused with them as in *L. dioica* ♂ and *L. alba* ♂ (fig. 11). In *L. coronaria* they arise practically independently (fig. 10).

Stamen traces arise independently from the stele, barely so in *L. alba* and *L. dioica*, markedly so in the other species. Staminodia in the pistillate flowers of *L. alba* and *L. dioica* show as small stumps or pegs, each with a short but distinct vascular trace (fig. 13). The antesealous traces arise slightly lower than antepetalous in *L. flos-jovis* (fig. 12), *L. alba* ♀, *L. dioica* ♀ (fig. 13), and *L. coronaria* (fig. 10); in the others there is no discernible difference. In all cases these strands lie in a single ring as soon as they have all become separate from the stele.

The stele closes for some distance above the stamen traces in all species; in *L. coeli-rosa* a short gynophore is externally visible (fig. 9).

Dorsal carpellary traces arise in a whorl of five on the sepal radii. Median laterals are lacking in *L. chalcadonica*, and arise in a single whorl some distance above the dorsals in *L. coeli-rosa* and *L. coronaria*. In the case of *L. flos-jovis* each dorsal trace branches into three (fig. 14A, B) and at the base of the loculi branches from adjacent dorsals fuse in pairs to form a set of five single traces which stand in the positions usually occupied by commissural median laterals (fig. 14C). In *L. alba* ♀ and *L. dioica* ♀, forms with large capsules, a set of minor freely-branching traces arises between the dorsals and the true median laterals. In all cases the median laterals end at the top of the loculi or the base of the styles.

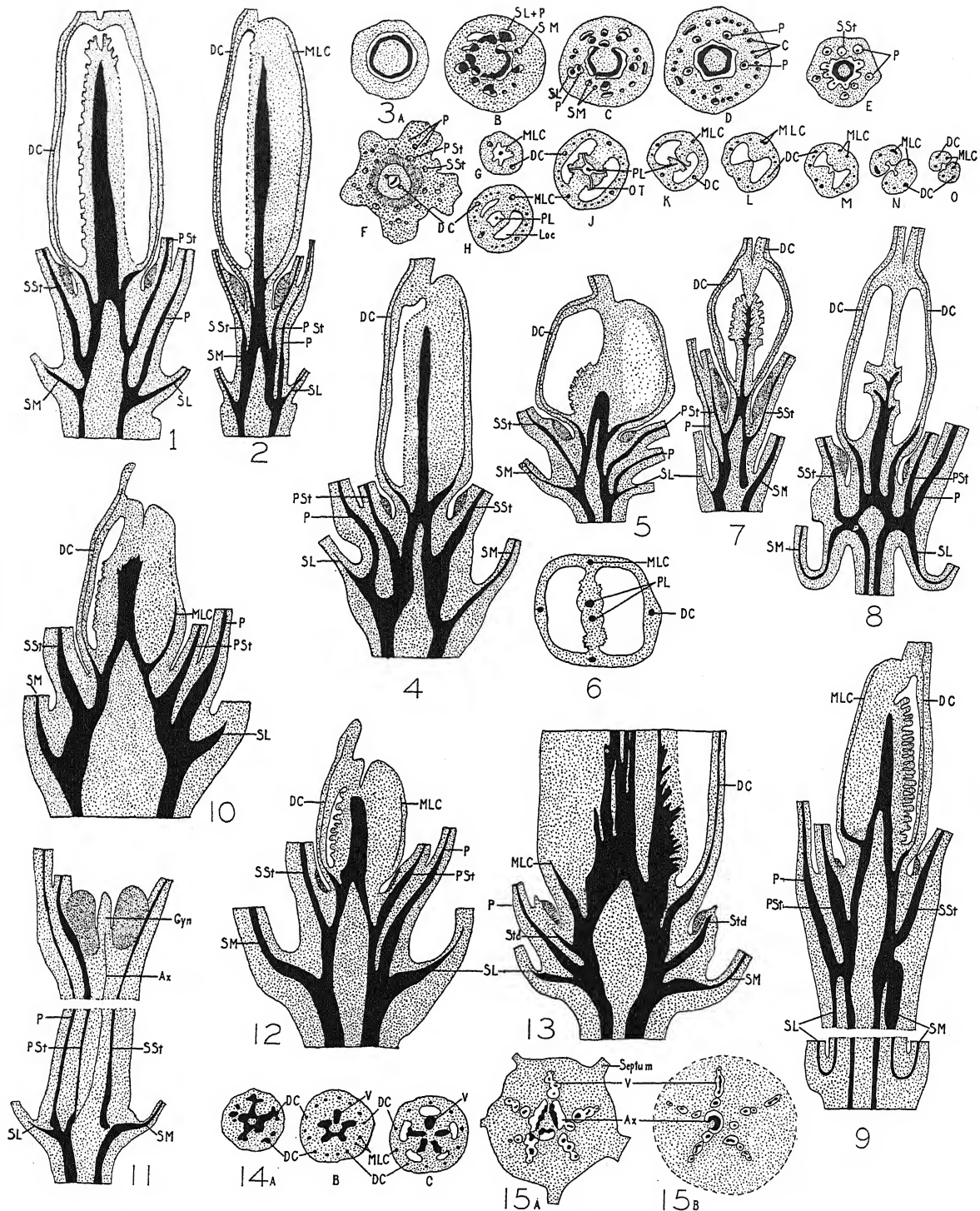


Fig. 1-15.—Fig. 1. *Dianthus chinensis*. Longitudinal section. $\times 7$.—Note: In figures 1, 2, 4, and 5 the gynoecium is represented in such a way as to show both dorsal and median lateral sections; actually the two halves of the gynoecium as shown should stand at right angles to one another. The rest of the flower is represented as it actually appears in section.—Fig. 2. *Dianthus barbatus*. Longitudinal section. $\times 7$.—Fig. 3. *D. barbatus*. Transverse sections from pedicel upward. A, pedicel just below flower. B, whorl of 10 calyx traces depart. C, large strands on petal radii split periclinally into inner petal trace and outer sepal lateral (commissural); sepal midrib trace splits. D, calyx traces divide; stele closes above perianth traces. E, calyx omitted; stamen traces arise, antesealous slightly lower. F, at base of gynophore; calyx omitted; petal traces split into three; antesealous stamen strands stand slightly farther out in recep-

The central vascular remnant consists of a complete cylinder, the pith of which is domed over in the base of the placenta. The vascular core above the pith has the form of a star, from the points of which arise the ovule traces. A strand of xylem elements forms an axis up the center of the placenta. In many sections, especially of *L. alba* ♀ and *L. dioica* ♀, the placenta shows a central vascular core with the arms or rays of the star detached from it and forming a ring around it. In such sections the core may form an almost complete cylinder with no gaps in front of the placental rays (fig. 15A, B). In these two species the xylem in the peripheral strands or rays is arranged as a central strand or a radial plate, as though the carpel edges met and fused, with adaxial faces together, but were not infolded (fig. 36). At the top of the loculi these vascular plates split radially and the adjacent halves fuse in pairs so that each septum as it withdraws from the center has one composite strand. These strands die out with the topmost ovule traces.

In *L. alba* ♂ (fig. 11) and *L. dioica* ♂ the gynoecium is represented by a parenchymatous finger with a central core of elongated, darker staining cells which seem to attach downward to the inner face of the vascular cylinder about at the level where the stamen traces originate.

L. dioica has been described by van Tieghem (1867), Henslow (1890), and Olbers (1895). These accounts agree with the observations presented above. Van Tieghem mentions the presence in the center of the placenta of vascular tissue which represents axis. In another work (1898, fig. 175f, p. 390) he shows a cross section of the placenta of *Lychnis* with five vascular rays as described above, and in the center of the placental column a ring of five vascular bundles with xylem toward the center. Lister (1883) figures a section of *L. dioica* (*L. diurna*) which shows a dark area, presumably vascular, in the center of the ring of arcs which represent the five placental bundles (Pl. 32, fig. 9). Dickson

(1936) shows a vascular patch in the center of the placenta in *Melandrium* (*L.*) *album*. Schumann (1889) declared that in *Melandrium* (*Lychnis*) *album* and *L. coronaria* the antepetalous stamens are inserted higher than the antesealous. Saunders' (1932) description of *L. chalcidonica*, *L. coeli-rosa*, and *L. flos-jovis* agrees with the present observations.

Olbers (1895) described *L. flos-cuculi* as having calyx traces arising in a whorl of ten, petal traces and antesealous stamen traces independent from the stele, antepetalous stamen traces fused at the base with petal traces, median lateral carpellaries lacking. *L. viscaria*, according to the same paper, is similar except that the calyx is saccate at the base and median lateral carpellaries are present and arise in a whorl above the dorsals.

Silene K 5, C 5, A 5 & 5, G 3.—Five species were studied: *S. viridiflora*, *S. pennsylvanica*, *S. noctiflora*, *S. pendula*, and *S. latifolia*.

The pedicel is traversed by a ring of five vascular strands which unite to form a short cylinder at the base of the flower.

The calyx is supplied by a single whorl of ten traces, five on midrib radii, the alternate five on petal radii. In *S. latifolia* the commissural traces branch into two or three strands immediately after leaving the stele. In this species and in *S. pendula* the base of the calyx is saccate or inflated, with the result that the sepal traces recurve sharply at the base (fig. 17).

The petal traces arise from the same ribs on the stele as the commissural calyx traces, but the two whorls are not really fused (fig. 18).

Stamen traces arise in a whorl of ten, antesealous ones independently from the stele, antepetalous ones from the same ribs as petal traces, except in *S. noctiflora*, where petal and antepetalous stamen traces are very slightly fused at the base (fig. 20). The outer tissues of petal and filament remain fused for some distance, often after the petals have separated from one another.

tacle; glandular ring on adaxial face of stamen bases; dorsal carpellary traces arising. G—O, gynoecium only. G, departure of median lateral carpellaries and extra traces for ovary wall. H, base of loculi. J, ovuliferous region showing placental strand with core of xylem, and attachment of ovule traces. K, top of loculi, septa pulling apart and placental trace split. L, M, juncture of ovary and styles; median lateral trace splits, septa bilobed at top. N, O, styles, showing dorsals and median laterals in styles, other traces died out. All $\times 14$.—Fig. 4. *D. armeria*. Longitudinal section. $\times 28$.—Fig. 5. *Gypsophila elegans*. Longitudinal section. $\times 14$.—Fig. 6. *G. elegans*. Section at top of ovary showing placental trace split just before septa pull apart. $\times 28$.—Fig. 7. *G. muralis*. Longitudinal section. $\times 28$.—Fig. 8. *Saponaria calabrica*. Longitudinal section. $\times 28$.—Fig. 9. *Lychnis coeli-rosa*. Longitudinal section. Portion of "internode" above calyx omitted. $\times 14$.—Fig. 10. *L. coronaria*. Longitudinal section of young flower. $\times 28$.—Fig. 11. *L. alba* ♂. Longitudinal section. Gyn=rudimentary gynoecium. One half of distance between calyx and base of glandular ring omitted. $\times 14$.—Fig. 12. *L. flos-jovis*. Longitudinal section. $\times 28$.—Fig. 13. *L. dioica* ♀. Longitudinal section showing traces to staminodes, and nature of placental strands. $\times 14$.—Fig. 14. *L. flos-jovis*. Sections through base of ovary showing origin of median laterals. A, dorsals fork into 3. B, ring of 15 strands destined for ovary wall, two strands between adjacent dorsals. C, at base of loculi wall contains 10 strands, one median lateral where there had been two. $\times 28$.—Fig. 15. *L. alba* ♀. Sections through placenta showing vascular tissue representing residual axis. A, base of loculi. Vascular tissue forms five radiating arms and a central cylinder with gaps. B, in ovuliferous region. Ovule traces supplied from ends of arms; stem residue forms an incomplete cylinder closed in front of most arms. $\times 14$.—SM=sepal midrib. SL=sepal lateral. P=petal trace. SSt=antesealous stamen. PST=antepetalous stamen. Std=staminode. DC=dorsal carpellary trace. MLC=median lateral carpellary trace. V=ventral carpellary strand. Ax=residual axis tissue. PL=placental strand (carpel and stem tissue not distinguished). X=xylem. OT=ovule trace. Loc=loculus. Glandular areas densely shaded. Where a distinction is made in the vascular bundles, dark area=xylem, unshaded=phloem, parenchyma, and procambial tissue.

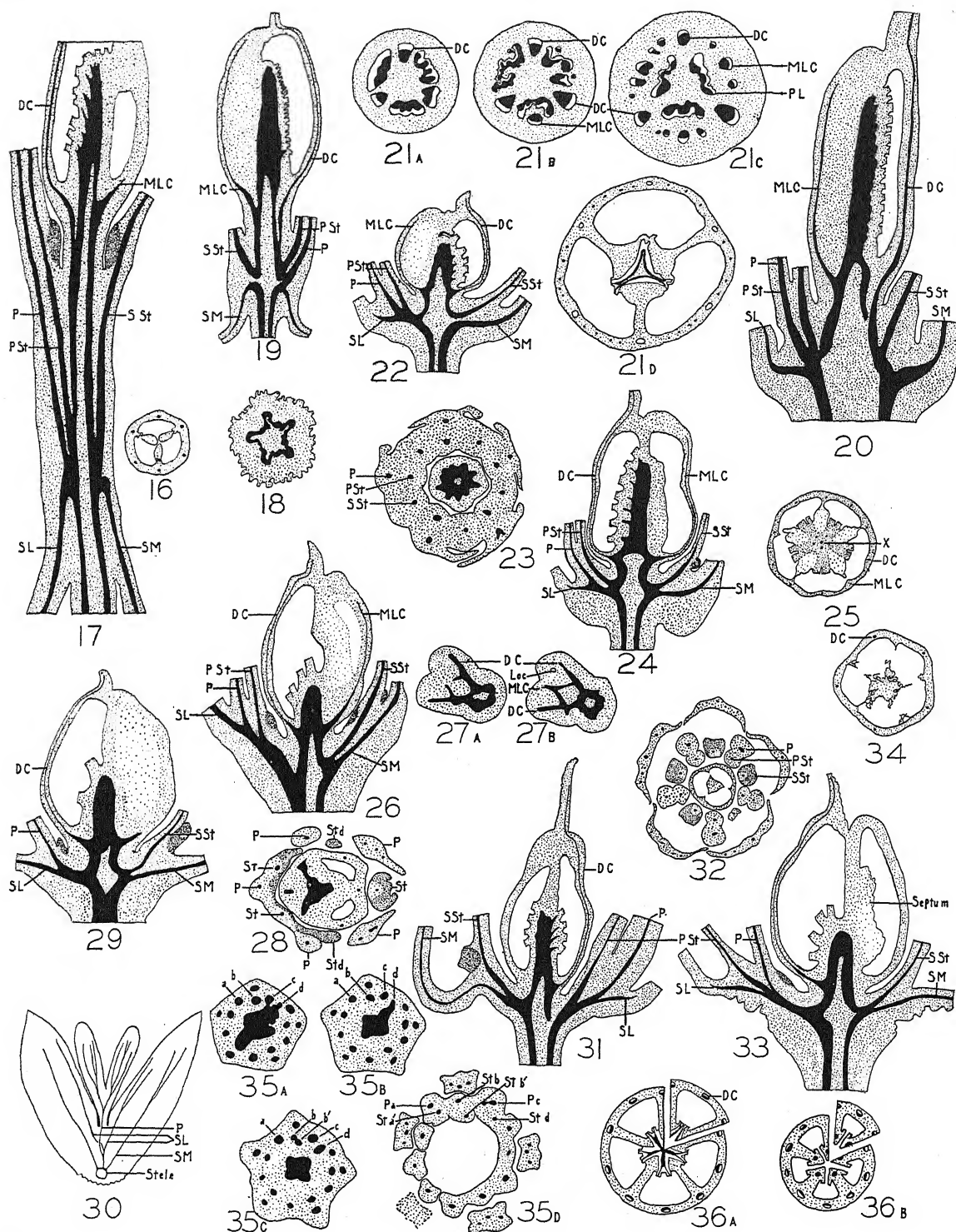


Fig. 16-36.—Fig. 16. *Silene pendula*. Upper part of capsule showing septa slightly withdrawn from center. $\times 14$.—Fig. 17. *S. pendula*. Longitudinal section. Septa torn. $\times 14$.—Fig. 18. *S. latifolia*. Transverse section of flower between departure of sepal traces and of petal traces showing stellar ribs. $\times 7$.—Fig. 19. *S. latifolia*. Longitudinal section. $\times 7$.—Fig. 20. *S. noctiflora*. Longitudinal section of young flower. Septa complete. $\times 28$.—Fig. 21. *S. latifolia*. Transverse sections of base of ovary. A, dorsal carpellaries separate. B, departure of median lateral and extra lateral traces. C, traces to ovary wall separated, placental supply consists of three arcs with xylem toward center. D, at base of loculi. Ventral supply forms hollow triangle, from points of which arise ovule traces. A-C $\times 12$, D $\times 7$.—Fig. 22. *Cerastium arvense*.

In *S. pendula* the whole base of the flower is much elongated (fig. 17); epidermal cells may be several times as long as they are wide in this region. A stelar internode occurs above the stamen traces; in *S. latifolia* (fig. 19) and *S. pendula* this is elongated into an externally visible gynophore.

The adaxial side of the briefly fused filament bases is glandular, forming a nectar cup around the base of the ovary.

In most species the stelar cylinder closes above the departing stamen traces, but in *S. viridiflora* the stele consists of six strands throughout this internode.

From the angles of the now triangular stele arise three dorsal carpellary bundles, which may branch in the ovary wall. The three strands left after the dorsals depart veer toward the center, flattening to form a hollow triangle which is usually open at the apices. From the abaxial face of each side a single median lateral carpel trace arises. This arrangement is complicated in *S. latifolia* by branching or dissection of strands and by the fact that the median laterals leave gaps (fig. 21). In *S. pendula* and *S. noctiflora* the median laterals bifurcate just before they die out at the top of the ovary. The vascular strands left after the departure of median lateral traces become the placental supply; this assumes the form of a hollow (*S. latifolia*) or solid triangle, from the attenuated points of which arise the ovule traces in pairs (fig. 21D).

S. noctiflora, *S. pennsylvanica*, and *S. viridiflora* show a small central strand of xylem elements in the ovuliferous region of the placenta. In *S. latifolia* and *S. pendula*, where the receptacular pith extends into the base of the placenta, the inner face of the stelar cylinder is occupied by a strip of xylem cells (fig. 21D). In the upper part of the ovary the placental vascular strand trifurcates, and shortly above this the septa separate and withdraw slightly from the center, as in *Dianthus et al.* (fig. 16).

Septa in young flowers (with immature ovules) are intact. The placental vascular tissue ends with the topmost ovule traces except for a small strand of provascular tissue continuing toward the top of each septum in *S. pendula*.

Dawson's (1936) description of *S. noctiflora* agrees with that given above. Henslow (1890) stated that in *S. inflata* (*S. latifolia*) petal and stamen traces are fused at the base; the material sectioned in the present study showed both sets of stamen traces arising directly from the stele. So far as could be determined from the figures given by Pratt (1932), *S. maritima* follows the plan described above for the family. Olbers (1895) described the vascular anatomy of *S. venosa* and *S. nutans*, in which the only difference from the rest of the family is that the antepetalous stamen traces arise lower than the antesepalous.

TRIBE ALSINEAE.—*Cerastium* K 5, C 5, A 5 & 5, G 5.—Two species were studied: *C. vulgatum* and *C. arvense*.

The pedicel shows a ring of five vascular strands, which closes to form a cylinder at the base of the flower.

A whorl of ten vascular traces departs from the stele. Of these, five become sepal midribs which branch variously in the base of the calyx. The alternate five branch anticlinally into three at a short distance from the stele. Of these three, the resulting lateral strands enter adjacent sepals as sepal laterals; the central strand immediately splits tangentially into petal trace and stamen trace (fig. 22). At the level of this last splitting a whorl of five stamen traces departs from the stele on the sepal radii, close above the sepal midrib traces. Sepal midribs become established in the base of the calyx a shade lower than do the laterals. The difference, however, is slight.

The fused soft tissues of filament and petal bases form a short tube, but the vascular strands for all members are separate and distinct from the base of the tube (fig. 23).

There is a short internode above the sepal-petal-stamen node.

At the base of the ovary arises a whorl of ten traces, the five on sepal radii becoming dorsal carpellaries, the alternate five becoming median laterals. The former extend into the styles, the latter end at the top of the loculi. The placental vascular supply forms a solid core with five lobes directed toward the loculi. This vascular core ends with the

Longitudinal section of young flower. $\times 28$.—Fig. 23. *C. arvense*. Transverse section through tube formed by petal and filament bases. Whorl of 10 carpel traces departing in ovary. $\times 28$.—Fig. 24. *C. vulgatum*. Longitudinal section. Septum torn. $\times 28$.—Fig. 25. *C. vulgatum*. Section through ovary showing complete but loose and fragmentary septa; central xylem strand surrounded by five small strands in placental rays. $\times 28$.—Fig. 26. *Stellaria graminea*. Longitudinal section. $\times 28$.—Fig. 27A and B. *S. aquatica*. Slightly oblique sections through base of ovary showing origin of median lateral carpellary traces. $\times 28$.—Fig. 28. *S. media*. Transverse section at insertion of stamens and petals showing three normally placed stamens (St) and two staminodes consisting of small glandular bumps (Std). $\times 28$.—Fig. 29. *S. media*. Longitudinal section. Septum complete but very fragmentary. $\times 28$.—Fig. 30. *S. media*. Petal with two adjacent sepals showing venation of calyx and corolla.—Fig. 31. *Arenaria groenlandica*. Longitudinal section. $\times 28$.—Fig. 32. *A. groenlandica*. Transverse section showing adherence of soft tissues of petals and filament bases; also distribution of glandular tissue. $\times 14$.—Fig. 33. *Spergula arvensis*. Longitudinal section. Septa fragmentary. $\times 28$.—Fig. 34. *S. arvensis*. Section of ovary above ovule attachment. Septa fragmentary but complete. $\times 28$.—Fig. 35. *Dianthus plumarius*. Transverse sections through base of flower showing origin of traces to extra petals and stamens. a and b, traces arising in usual way; c and d, extra traces direct from stele. (35C. b'=extra trace derived by splitting of b. 35D. Pa, Sta'=petal and stamen traces derived by splitting of a; Stb, Stb'=stamen traces by splitting of b; Pc=extra petal trace arisen directly from stele; Std=extra stamen trace arisen directly from stele.) $\times 7$.—Fig. 36. Diagrammatic representation of *Lychnis* ovary, one carpel loosened and slightly opened along ventral face. A, at level of ovule attachment. B, above ovules.

TABLE 1. Summary of anatomical data on the Caryophyllaceae.

	<i>Dianthus armeria</i>	<i>D. barbatus</i>	<i>D. chinensis</i>	<i>D. plumarius</i>	<i>Gypsophila elegans</i>	<i>G. muralis</i>	<i>Gypsophila</i> sp.	<i>Saponaria calabrica</i>	<i>Saponaria</i> sp.	<i>Lychnis alba</i> ♂	<i>L. alba</i> ♀	<i>L. dioica</i> ♂	<i>L. dioica</i> ♀	<i>L. chalcidonica</i>	<i>L. flos-joris</i>	<i>L. coeli-rosa</i>	<i>L. coronaria</i>
Pediceel:																	
Complete cylinder	×	×	×	×	×	×	×	×	×	×	×	..
Ring of five strands	ca.	ca.	8	8	×	×
Ring of ten strands	×
Calyx:																	
Midribs below commissurals ..	×	sl. ^a	..	×	×	×	×	×	..	×	×
All traces in one whorl	×	×	×	×	×	×	×	×	×	×	×	×	(X)	×	×	..
Calyx saccate at base	×	×	×	..
Petals:																	
Traces independent from stele	(sl.)
From same ribs as commissurals	×	×	×	×	×	×	..	(sl.)
Fused at base with commissurals	×	×	×	..	sl.	sl.	sl.	×	×	sl.	..	sl.	×	..
Stamens:																	
Two cycles present	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
All arise on same level	×	×	×	×	×	..	×	..	×	..	×	..
Antesepalous arise lower	×	×	×	×	×	..	sl.	..	sl.	..	sl.	..	sl.
Antesepalous stand outer	sl.	×	×	×
All arise independently	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Antepetalous fused with petal trace	×	×
Antesepalous fused with sepal trace	sl.	sl.
One cycle: antesepalous
Soft tissue fused with petal tissue	×	×	×	..	×	×	×
Carpels:																	
Gynophore present	×	×	×	×	sl.	×	×	×	sl.	..	sl.	×	..
Dorsals antesepalous	×	..	×	×	×	×	×
Dorsals antepetalous
Median laterals:																	
Arise above dorsals	×	×	×	×	×	×	×	×	sl.	..	×	..	×	×	×
In whorl with dorsals	×
Branch from dorsals	×
Lacking	×
Fork, enter styles	×	×
Fork, die out	×	×	..	×	×	×	..
Placenta with central xylem strand	×	×	×	×	..	×	×	×	..	×	×	×	×	..
Pith continuous from receptacle to above median lateral carpellaries	sl.	..	sl.	×	..	×	..	sl.	×	×
Glands:																	
Ring on adaxial stamen bases	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	..
Surrounds bases of antesepalous filaments
On abaxial side of antesepalous filaments

^a Note: sl.=slightly.

ovule traces. In *C. vulgatum* a central strand of one to three xylem elements is surrounded by five small xylem strands in the placental lobes. The whole central area of the placenta is procambial, with indistinct limits. The ovary is completely partitioned, but the septa are fragmentary and loosely woven from an early stage. The placenta is large and round in cross-section, and fragmentary except for the five arms or radially arranged plates to which the ovules

attach (fig. 25). Styles are set back from the center of the ovary—that is, they stand above the loculi rather than above the placenta.

Saunders (1932) states that in *C. arvensis*, *C. chloeraefolium*, and *C. alpinum* the vascular bundles for sepals, petals, and two stamen whorls turn out as one whorl. This would mean that antesepalous stamen traces arise fused with sepal midribs. The material sectioned in the present study shows antesepalous

TABLE 1. *Concluded.*

	<i>Silene latifolia</i>	<i>S. caroliniana</i>	<i>S. noctiflora</i>	<i>S. pendula</i>	<i>S. viridiflora</i>	<i>Cerastium vulgatum</i>	<i>C. arvense</i>	<i>Stellaria media</i>	<i>S. graminea</i>	<i>S. holostea</i>	<i>S. aquatica</i>	<i>Arenaria groenlandica</i>	<i>Spergula arvensis</i>	<i>Sagina procumbens</i>
Pedicle:														
Complete cylinder	×	×	..	×
Ring of five strands	×	×	×	×
Ring of ten strands	8	×	×	..	×	×	2	..
Calyx:														
Midribs below commissurals	sl.	×	×	×	×	×	×	×
All traces in one whorl	×	×	×	×	×	×	×	×	×	×	×	×	×
Calyx saccate at base	×	×	×
Petals:														
Traces independent from stele
From same ribs as commissurals	×	×	×	×	×
Fused at base with commissurals	×	×	×	×	×	×	×	×	×
Stamens:														
Two cycles present	×	×	×	×	×	×	×	±	×	×	×	×	(×)	±
All arise on same level	×	×	×	×	×	×	×
Antesepalous arise lower	sl.	×	sl.	×
Antesepalous stand outer
All arise independently	×	×	..	×	×	sl.
Antepetalous fused with petal trace	sl.	sl.	×	..	×	×	×	×	×	×
Antesepalous fused with sepal trace	×	×	..	×	×	×
One cycle: antesepalous	(×)	(×)
Soft tissue fused with petal tissue	×	sl.	×	×	..	×	sl.	×	×
Carpels:														
Gynophore present	×	..	sl.	×	sl.	sl.	sl.	sl.	sl.
Dorsals antesepalous	×	×
Dorsals antepetalous	×	..	×	×	×
Median laterals:														
Arise above dorsals	×	×	×	×	×	sl.	×
In whorl with dorsals	×	×	..	×
Branch from dorsals	×
Lacking	×	×	×	×
Fork, enter styles
Fork, die out	×	×
Placenta with central xylem strand	×	×	..	×	×	×	×	×
Pith continuous from receptacle to above median lateral carpellaries	×	×	×	×	sl.	sl.	sl.	..	sl.	..	sl.	..	×	..
Glands:														
Ring on adaxial stamen bases	×	×	×	×	×	sl.	×	sl.	sl.	×
Surrounds bases of antesepalous filaments	×	×	..	×	×	sides	×
On abaxial side of antesepalous filaments	×	..	×	×	×	..	×

stamen traces arising independently, although they stand close above the sepal midribs (fig. 22, 24).

Stellaria K 5, C 5, A 5 & 5 to 3, G 3.—Four species were studied: *S. media*, *S. graminea*, *S. holostea*, and *S. aquatica*.

Except for the gynoeceium of *S. aquatica* the species of *Stellaria* examined are essentially similar in vascular pattern to *Cerastium*. Differences found are:

1. The petal-sepal lateral-antepetalous stamen strand is less massive at its origin. The sepal laterals depart from the petal strand farther from the stele than does the antepetalous stamen trace (fig. 29).

2. Antesepalous stamen traces are slightly fused with sepal midribs in *S. graminea* and *S. holostea* (fig. 26).

3. The dorsal carpellary bundles arise slightly lower than the medians in *S. media* and *S. graminea* (fig. 26, 28, 29).

4. The placental or ovuliferous column is somewhat shorter in proportion to the length of the capsule in most cases.

5. Styles stand over the placental column as in most genera.

6. The ovary of *S. aquatica* differs from the others examined. It consists of five carpels rather than three, and the loculi are antepetalous, thus breaking the regular alternation of whorls. Dorsal carpellaries arise in a whorl of five, each of which branches into three near its base. The side branches from adjacent dorsals fuse below the loculi; these fused bundles become median carpellaries (fig. 27A, B).

In this species the general form of the capsule is more like *Cerastium* than like other *Stellaria* species in that it is more elongate and that almost the entire placental column is ovuliferous.

In the highly variable *S. media* no vestigial vascular traces were found at the site of missing stamens. The five-stamen form is like a ten-stamen flower, as of *S. media neglecta*, with the antepetalous stamens completely removed. In forms with less than five stamens the remaining stamens stand in three or four of the normal stamen positions (fig. 28). The only trace of a missing member is a slight bump frequently present on the glandular ring at the site of the deleted stamen.

According to Wester (1899) the calyx midribs of *S. graminea* arise slightly lower than the laterals; otherwise her description of this species and of *S. (Malachium) aquatica* agrees with the writer's observations. Stroebel (1925) declared that *S. holostea* is obdiplostemonous by ontogeny and by insertion. He gave no illustrations nor any more specific information on this point.

Peterson (1936) studied chromosome numbers in the *S. media* group and concluded that *S. neglecta* is the point of departure for the morphologically reduced forms; the latter are considered autotetraploids with a few chromosomes missing from the 2n number in some cases.

Arenaria K 5, C 5, A 5 & 5, G 3.—One species was studied: *A. groenlandica*.

A whorl of ten traces leaves the central cylinder at the base of the flower. At a short radial distance from the stele each of these ten traces splits tangentially. Strands resulting on sepal radii become a sepal midrib and an antesealous stamen trace; of those on the petal radii, the inner becomes an antepetalous stamen trace, the outer subsequently branches into three, of which the middle member becomes a petal trace and the laterals become marginals for adjacent sepals (fig. 31).

The soft tissues of sepal, petal, and filament bases are fused into a short tube or cup about the base of the ovary. The filaments of the antepetalous stamens remain joined to the petals after the petals have separated from one another (fig. 32).

Dorsal carpellary strands arise as a whorl of three. Median lateral bundles are lacking.

The relatively long ovary is completely septate, but the partitions early become fragmentary. The placental column is complete from base to apex of the loculi, but ovules are borne on only the middle third of this column (fig. 31). The placenta is traversed by the usual slender core of xylem cells.

Wester's (1899) description of *A. trinervia* and *A. (Alsine) biflora* agrees with the account given above. Mattfeld (1921) reported that the ovary of *A. (Minuartia) scleranthus* in the young flower is actually sunken in a cavity in the top of the axis. As the capsule grows, it protrudes, but the base remains enclosed by the axis and may be constricted by it.

Spergula K 5, C 5, A 5 & 5, G 5.—One species was studied: *S. arvensis*.

The pedicel bears two or three strands which are separated only by narrow strips of parenchyma and form an almost solid cylinder. Calyx, corolla, and stamens are supplied as in *Arenaria*. Glandular tissue forms a ring on the adaxial face of this tube near its base and extends to the sides of the filament bases.

Of six flowers sectioned transversely, one had only the antesealous stamen whorl developed. In this case no indication of another whorl could be found, either as humps of parenchyma or glandular tissue or as vestigial vascular traces.

After a short stelar internode, a whorl of five dorsal carpellary traces departs on the petal radii. These give rise to various small branches in the ovary wall. Real median laterals are lacking. The placental vascular supply consists of a slender central cylinder with five arms, radiating toward the dorsal carpellaries, from which the ovule traces are supplied. The central pith dies out about halfway up the ovuliferous column. The radial plates or arms of vascular tissue appear in some sections to be detached from the central strand, as in the case of *Lychnis* described above. Toward the top of the placenta all these strands become small and scattered and finally die out.

In young flowers septation of the ovary is complete, although the septa early become loose and fragmentary (fig. 34). The ovuliferous region of the placenta extends about a third of the way up into the capsule.

These observations agree with Wester's (1899) description of the species and with Saunders' (1932) brief mention of it.

Sagina K 4, C 4 (or less), A 4 & 4 (or less), G 4.—One species was studied: *S. procumbens*.

The pedicel is traversed by a complete vascular cylinder.

In the base of the flower a whorl of eight traces leaves the central cylinder. These are distributed to calyx and corolla as in *Arenaria*. In flowers with complete sets of petals and stamens, at the separation of sepal laterals, there is given off a central trace which immediately splits tangentially into petal and antepetalous stamen traces. The number, position, and degree of development of petals and stamens varies widely in this species. Of six flowers sectioned transversely, five had four antesealous stamens and the other had two. Petals and antepetalous stamens may appear as fully formed organs; or as small scales or bumps with very faint and abbreviated vascular traces or no traces at all; or they may be completely lacking with no discernible vestiges. Petals occur somewhat more frequently than antepetalous stamens, but either member may occur at a given corner of the flower.

"Perigyny" is developed to about the same degree as in *Arenaria groenlandica* (fig. 31). In *Sagina* the stamen bases form a short ring or tube above the separation of stamens from petals.

In the short internode below the ovary the stelar cylinder becomes slightly four-lobed. At the base of the ovary a whorl of four dorsal carpellary traces

departs from the central cylinder on petal radii. Above the separation of the dorsals, the placental vascular strand is round in cross-section, becoming X-shaped higher up in the placenta. Ovule traces attach to the arms of the cross. Vascular tissues are weakly lignified and xylem cells difficult to identify in cross section. So far as could be determined, the placenta bears a thin central core of xylem cells.

The placental column extends the entire length of the capsule, but the top third bears no ovules. In the youngest material sectioned (proembryo stage) separation is incomplete in the upper region of the capsule. Ridges occurring on the commissural lines of the ovary wall and on the placenta opposite, between the double rows of ovules, show no sign of tearing or fragmentation. According to Lister (1883) the septa cease vertical growth earlier in development than the ovary wall, so that the capsule is at no time completely septate.

Saunders (1932) shows a cross-section of the gynophore of *S. procumbens* (fig. 51) with eight vascular bundles. The material studied here shows a four-sided cylinder of darkly staining cells with some faintly lignified xylem elements in the center. The dorsal carpellary bundles arise from the angles of this cylinder.

The above observations agree with those of Dawson (1936) on the same species. *S. nivalis* (Wester, 1899) is a pentamerous flower but otherwise shows the same vascular plan.

The entire family is characterized by sepal lateral veins which arise commissurally—that is, as single strands (representing two laterals of adjacent sepals) on the petal radii which show no indication of doubleness until they fork into pairs of marginal veins. These arise in close association with the petal traces. Fusions between whorls do not affect the carpels; in all species examined, a definite stelar internode separates dorsal carpellaries from the traces of lower whorls. Median lateral carpellary traces arise commissurally and show scant sign of doubleness. The two ventral traces for each carpel are completely fused, and in most cases all ventrals are fused into a single vascular core in the placenta. Most of the species examined show a solid column of xylem cells in the center of the placenta; the diameter of this varies with the size of the capsule, but the xylem may be present even in the smallest flowers. In the upper part of the ovary septa do not meet in the center, as though the carpel margins do not quite meet for a part of their length.

The two tribes may be briefly characterized as follows:

Sileneae.—Gamosepaly ranging from simple adherence of soft tissue to fusion or anastomosing of vascular systems; a tendency for all traces supplying perianth parts to form a single whorl, and for all stamen traces to form a single whorl without a reduction in number; stamen traces independent of traces of other whorls; frequent occurrence of a gynophore; indications of incomplete carpel fusion.

Alsineae.—Sepals separate; “perigyny” by fusion of sepal lateral-petal-stamen strands and tissues, and fusion between sepal midrib traces and antesepalous stamen traces in smaller forms with the result that only two whorls of traces depart from the stele to supply all floral members; variability and reduction in number of petals and stamens; sometimes a change in alternation sequence with reference to carpels; progressive reduction in size of whole flower, of ovuliferous region of placenta, and of venation in carpel wall.

Details are summarized in table 1.

DISCUSSION.—The anatomy of the species here investigated shows a progressive telescoping of whorls along the floral axis, affecting first the lowest whorls. The sepal is a three-trace organ as in the leaves of most Centrospermae (Sinnott, 1914). The laterals of sepals arise commissurally throughout the family, as in many angiosperms in widely scattered families. In a few cases among the *Sileneae* the sepal midribs arise lower than the commissurals, as in *Dianthus armeria* and some species of *Lychnis*, but in most species the two sets of traces arise at the same level. The next stage of condensation is adherence between sepal commissurals and the superposed petal trace. In *Lychnis* and *Silene* these two whorls are separated by a short vertical distance, but arise from the same ridges or ribs on the stele (fig. 18). In other genera there is actual fusion of the basal parts of the superposed traces.

The internode that is externally apparent between calyx and corolla is due to adherence of a saccate calyx base to the pedicel which it surrounds. Anatomically there is no internode here.

Traces for antesepalous stamens arise lower than those for antepetalous in *Dianthus* and *Saponaria*, less so in scattered species of *Silene* and *Lychnis*. In other forms the two sets are contracted into a single whorl. In *Saponaria* and the *Alsineae*, the antepetalous stamen traces arise fused with the petal traces; in some species of *Stellaria*, in *Arenaria*, *Spergula*, and *Sagina* those for antesepalous stamens are fused with sepal midribs. Anatomically none of the species examined is obdiplostemonous. Fusions between perianth and stamen traces are cumulative, that is, if antesepalous stamen strands are fused with sepal midribs, then antepetalous are always fused with petal strands and with calyx commissurals.

Fusion between whorls does not affect the carpels. The ovary is elevated upon a rather short gynophore in *Dianthus*, *Gypsophila*, and a few other *Sileneae*, but a real stalk is never developed in the *Alsineae*. In all species there is a vascular internode between the departure of stamen traces and that of carpel strands. Within the carpel whorl some telescoping is apparent in that the dorsals consistently arise at a lower level than the laterals in the *Sileneae*, but all form a single whorl in many members of the other tribe.

Median lateral traces branch from the dorsals and fuse in pairs, as described above, in the ovaries of *Lychnis flos-jovis* and *Stellaria aquatica*. In other

species these medians arise commissurally, but separation of the component strands occurs at the top of the loculi in many Sileneae. In *Dianthus barbatus* and *D. chinensis* the medians enter the styles, but in other species the forks soon die out. In some Sileneae and all Alsineae the strands die out without forking.

The vascular supply to the ovary wall varies with the size of the capsule. Large capsules bear an extra set of strands between dorsals and median laterals as in *Dianthus barbatus*, *D. plumarius*, and *D. chinensis*, and *Lychnis alba* and *L. dioica*. Small capsules as in *L. chalcedonica*, *Arenaria*, *Spergula*, and *Sagina* develop no medians at all.

In flowers with an isomerous gynoeceium the carpels may be antesepalous, as in *Lychnis* and *Cerastium*, or antepetalous as in *Agrostemma*, *Stellaria*, *Spergula*, and *Sagina*. Since all of these genera bear five whorls of floral parts, of which the outer four arise in acropetal sequence (Payer, 1857) and stand on alternating radii, this variation in location of the carpels has puzzled many morphologists. Explanations based on dedoubement or omission of perianth or stamen whorls are not substantiated by ontogeny nor by anatomy of mature flowers; moreover, such theories may explain one position of carpels or the other, but not both at the same time. Alexander Braun suggested that two whorls of carpels are potentially present, but one is always missing (Eichler, 1875, p. 111).

Hofmeister (Čelakovský, 1875) postulated that primordia arise where there is a gap or an unoccupied space. Čelakovský (1875) studied the problem of obdiplostemony and suggested that in flowers such as those of the Caryophyllaceae with antepetalous carpels the antepetalous stamens have become phylogenetically displaced downward on the axis, so that at the time of carpel initiation the gaps on the axis are antepetalous. Schumann (1899) decided that in *Malachium* (*Stellaria*) *aquaticum* the antepetalous stamens are probably higher than the antesepalous, and that contact at the time of formation is the decisive factor in carpel position.

Saunders (1935) suggested that in flowers where the midribs for all whorls arise directly from the stele, after formation of the second stamen whorl a "state of congestion" is induced. By the time of recovery from this state the spirally advancing "growth impulse" has passed to the petal radii, and carpels arise there. *Agrostemma* has been described by Olbers (1895) as having stamen traces arising in two whorls independently from the stele. So far as I can determine from the literature, a gynophore is no more highly developed here than in *Lychnis*. Other species showing a gynophore have oligomerous gynoecea and are thus not comparable. In both *Stellaria aquatica* and *Cerastium* the antepetalous stamen traces are fused with petal traces, antesepalous arise directly from the stele; the length of the stelar internode above the stamen trace whorls is about the same in both cases. The cause of the discrepancy in position of carpels in these forms is thus not revealed by anything in their mature anatomy. Perhaps a

comparative histogenetic and anatomical study would reveal some pertinent factors.

Stellaria, *Spergula*, and *Sagina* often bear only one stamen whorl. In such cases the alternation sequence of whorls is regular and there is no problem of symmetry. Perhaps spatial relations have some influence on the dropping out of whorls in this family. On this basis it is easy to see why the antepetalous stamen whorl disappears before the antesepalous in these genera; but the cause of the original shift in carpel position remains unexplained.

Nature of the floral parts.—The facts here presented throw no new light on the nature of sepal or stamen. The sepal appears to be a three-trace leaf-like structure, the stamen a one-trace autonomous member. The ephemeral existence of the stamen may be the cause of its weak vascularization. The vascular strand in the filament usually consists of only a few xylem elements in the center of a slender cylinder of parenchyma, phloem, and procambial tissue. The organization of this strand may be truly radial, or it may be that cells on the adaxial side of the bundle never differentiate into mature xylem elements because of the brief life of the stamen. In the light especially of paleobotanical knowledge, Wilson's (1937) concept of the stamen as a reduced branch system comparable in its origin to the angiospermous foliage leaf seems the most tenable idea yet proposed.

The petal has been interpreted as a leaf, as fused stipules of a stamen, and as a metamorphosed stamen. Nothing observed in this study suggests a direct origin from a leaf or a sepal. The texture and venation are distinctly unlike those of leaf and sepal. Anatomically the petal does not resemble a pair of stipules. If the petal is composed of stipules and is homologous with a nectar gland, then stipules of antesepalous stamens are never vascularized while those of alternisepalous stamens always are; the traces for stipules are always fused below the midrib, and so completely fused that the vascular system first branches on a plan of three instead of two; and in the Sileneae the antesepalous stamens have no stipules and are inserted in some species at a level between the alternisepalous stamens and their stipules. The facts can be explained much more simply and with less stretching of facts to fit the argument if petals are considered independent and autonomous organs.

Several substantial facts support the concept of petals as metamorphosed stamens. Extra petals formed in double flowers show a close series of transition forms from stamen to petal (Smith, 1927). Troll (1927) has reconciled the form of petals to their origin from stamens. In double flowers such as cultivated forms of *Dianthus* or *Gypsophila* the vascular strand for an extra member may arise by splitting of a normally placed strand or depart directly from the stele; both situations may occur in the same flower (fig. 35A-D). The extra member supplied by a splitting stamen trace may develop as a stamen or as a petal. The vascular supply for petals consist-

ently arises as a single strand, as in the case of stamens. The tendency to dichotomy in petal venation may indicate a secondary elaboration in response to the needs of an expanded organ.

In general form the carpel resembles a leaflike organ rolled longitudinally, so that the edges meet at top and bottom but are separated for a short distance near the top in the region where the septa withdraw from the center. The bilobed form of the free ends of the septa in this region in *Dianthus armeria*, *D. barbatus*, and *Saponaria calabrica* further suggests the slight separation of two folded laminar organs. Despite the description generally given in taxonomic works of the pistil as unilocular, the capsule is distinctly septate, at least when young.

Anatomically the carpel is a five- or three-trace member in which median laterals of adjacent carpels have coalesced more or less completely. Throughout the family the ventral carpel traces are fused in pairs, forming strands standing opposite the dorsal trace. Often the entire ventral supply forms a central mass in the placenta. In most species the tissues can be roughly allotted to specific carpels because of the arrangement of the placental vascular tissue in rays or arms, each standing opposite a dorsal carpellary bundle and each supplying a double row of ovules; but in the lower portion of the ovary there is often no real demarcation of the separate bundles of which this placental mass is presumably composed. Higher up, where the septa pull apart from the center, the ventral traces separate (fig. 36), one standing in the edge of each septum and each probably composed of the strands of adjacent carpels which have coalesced.

The presence of some residual stem tissue in the ovuliferous region of the placental column is indicated by the central vascular cores described above in *Lychnis* (fig. 15A, B) and *Spergula*. This feature has been figured by several writers but ignored beyond the indication of it in their drawings, except for van Tieghem, who considered it as stem tissue, but of no great significance. The persistence of a xylem strand in the center of the placental column (fig. 25) in even the most reduced forms such as *Arenaria* and *Sagina* suggests that a slight remnant of stem tissue is present. The central pith may be continuous from receptacle to above the departure of median lateral carpel traces, as in all *Lychnis* species examined, some *Silene*, some *Gypsophila*, *Spergula*, *Cerastium*, and some *Stellaria*. Schaefer (1890) suggested that the pulling apart of septa in the upper part of the ovary indicates the level at which the stem ends. Lister proposed that the rudimentary structure in the position of pistil in staminate flowers of *Lychnis alba* and *L. dioica* might be the stem apex which forms no carpels.

All the evidence taken together points to the presence of some residual axis tissue in the center of the placental column. This has been definitely established for some ranalean species (Eames, 1931), and there seems to be no reason for discrediting this interpretation in this family. It does not affect the true

appendicular nature of the placentae, since the stem residue is in excess of the tissues composing the carpels. The presence of such a residue is another aspect of the reduction from large primitive forms.

Since the telescoping described affects the lower or first-formed whorls first, the limitation of growth or reduction in length of the axis must be determined at the very start of floral development.

Newman (1936) considered the carpel of *Acacia* to be a lateral organ; although the amount of tissue representing axis is very small, it can be followed through at least the early stages of carpel formation. McCoy (1940) in a study of the floral histogenesis of *Frasera carolinensis* found that sepals, petals, stamens, and carpels all arise in a similar manner from two distinct superficial and a third inner "germ layers." Brooks (1940) reported that the floral apex in *Amygdalus communis* differs from the vegetative in possessing only one tunica layer rather than four, and that the carpel differs from the other floral parts in that the entire floral apex is involved in its formation. Satina, Blakeslee, and Avery (1940) grew periclinal polyploid chimaeras of *Datura* in which germ layers can easily be distinguished by the characteristic cell size of each layer. Histogenetic studies on such flowers should reveal interesting data, especially on the later developmental stages which are difficult to follow in flowers of homogeneous composition.

Relationships as indicated by floral anatomy.—Within the family the species investigated fall into groups that agree with the general taxonomic treatment. The two tribes Sileneae and Alsineae differ in anatomy as in external characters. Among the former, *Lychnis* and *Silene*, sometimes designated as Lychnidae, are closely similar in the venation of the calyx, where commissurals are fused to a point just below the sinuses, and branches from midribs and from commissurals often anastomose distally; petal traces arise in close conjunction with calyx commissurals, but are in most cases not really fused. *Dianthus*, *Gypsophila*, and *Saponaria* form another closely similar group, sometimes referred to as the Diantheae. In this group, branches from calyx commissurals do not anastomose with midrib branches; petal traces are actually coalesced with calyx commissurals at the base; a gynophore is present in most species; median lateral carpellaries fork at the top; carpels are reduced to two. The two groups probably diverged from a common ancestral form, *Lychnidae* advancing further into gamosepaly and syncarpy, Diantheae showing further adhesion in the perianth, oligomery in the gynoeceum, and reduction of axial residue in the pistil.

The Alsineae can be regarded as a single line, uniform in the vascular pattern of calyx and corolla, with progressive adhesion of stamen whorls to perianth leading to slight but definite "perigyny," and showing progressive reduction in the gynoeceum.

Reduced forms are regarded as derived on the grounds that oligomery, anatomical fusion, "peri-

gyny," and reduction of residual axis tissue are all characters which show increasingly wide departure from the primeval form of flower. This view is further supported by the work of Kraft (1917), who reported that in the course of their ontogeny many species exhibit actual reduction in the number of members by abortion or resorption of primordia; and by that of Matzke (1932) showing that oligomerous flowers in *Stellaria media* most often exhibit radial or bilateral symmetry, but that this symmetry is limited by the disposition of parts in the most complete ground plan.

Further reduction from forms such as the more reduced Alsineae leads to the Paronychieae, which merge into reduced Centrosperms families such as the Chenopods and Amaranths.

The anatomical facts here presented support the view of Douglas (1936) and Dickson (1936) that the Primulaceae may be derived by further reduction of the septa from Caryophyllaceae. In both families traces for calyx and corolla are closely related; stamens show a tendency to epipetaly by adhesion of soft tissues in the Sileneae, and in the Primulaceae the vascular strands for these two whorls also coalesce; the vascular plan in the gynoecium is similar in the two families, although the ventral strands of Caryophyllaceae often show coalescence into a placental mass. Dickson (l. c.) concluded that residual axis tissue is present in the placenta, but no specific vascular tissue could be allotted to the axis; the Primulaceae thus show a variation from the Caryophyllaceae in further reduction of residual stem tissue.

The derivation of the Caryophyllaceae from the Geraniaceae as suggested by Dickson is less convincing. The latter family is characteristically obdiplostemonous with antepetalous carpels. The arrangement of stamens can be explained by the occurrence in some forms of vestigial traces of a third and outermost stamen whorl standing just above the sepals. Traces for stamen and perianth whorls show fusions similar to those occurring in Caryophyllaceae and Primulaceae, but there is no indication of a third stamen whorl in either of these two families. The Caryophyllaceae were shown above to be truly diplostemonous. The number of floral whorls as fixed in primitive ancestral forms would seem to be a more fundamental test of relationships than the occurrence of similar types of vascular fusions arising

presumably as a result of shortening of the floral axis, which happens in all floral lines.

The Polemoniaceae (Dawson, 1936) show a fundamental vascular plan comparable to that of Primulaceae and Caryophyllaceae. Noteworthy is the occurrence of a central xylem strand in the placenta of many polemoniaceous species, as well as in many of those here investigated. This has not been reported for other groups. The most striking deviation of the Polemoniaceae from the other families is the lack in this group of a tendency to suppress carpelary septa. The three families may be regarded on the basis of their floral anatomy as parallel lines, possibly stemming from a common ancestor, with the Polemoniaceae diverging before the weakening of septa and attaining a comparable degree of reduction and fusion.

SUMMARY

The vascular anatomy of the flower is described for twenty-nine species in ten genera of the Caryophyllaceae.

The anatomy of the species investigated shows progressive telescoping with consequent coalescence of whorls and their vascular traces along the floral axis, affecting first the lowest or outermost whorls.

Explanations of the inconstant location of the carpels are discussed.

Anatomical data support the concept of the petal as a metamorphosed stamen, of the carpel as a folded leaflike structure. The capsule in flowers up to the stage of anthesis is completely septate except in the case of *Sagina*. Vascular tissue representing residual axis tissue is present in the placenta, especially in the Lychnidae. Ventral carpel traces are coalesced into a central placental mass or into a group of separate strands.

The anatomy of the species investigated supports the usual taxonomic treatment of relationships within smaller groups in the family. The Alsineae are a more reduced and anatomically more advanced group than either section of the Sileneae.

The relationship of the Caryophyllaceae to the Primulaceae is upheld, but direct relationship to the Geraniaceae seems improbable, and to the Polemoniaceae remote.

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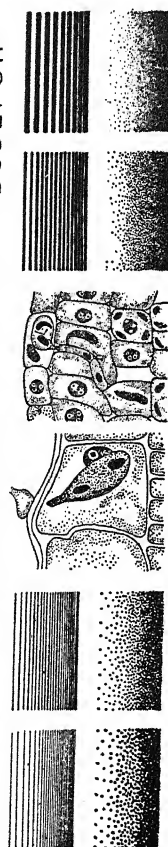
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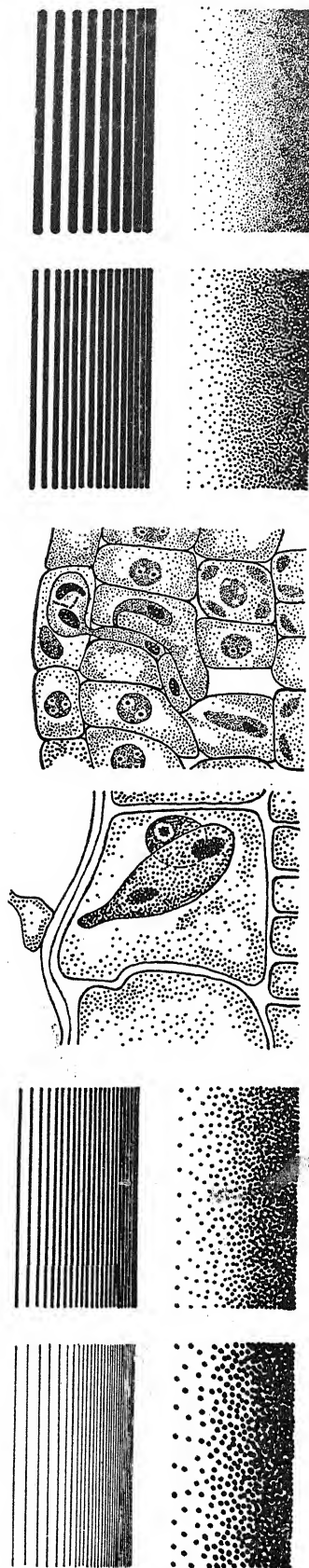


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POPULATIONS OF LINARIA ON THE GULF COAST ¹

Norman C. Fassett

ABOUT PENSACOLA, Florida, occur all three of our native North American species of *Linaria* (Pennell, 1935, maps 82-84). These are: *L. texana* Scheele, which is widespread west of the Mississippi River but eastward is confined to the southern states; *L. canadensis* (L.) Dumort, common eastward, especially on the Atlantic Coastal Plain, and of scattered occurrence west of the Mississippi River; and *L. floridana* Chapman, of peninsular Florida and the Gulf Coast, with one record from the interior of Georgia.

In April, 1941, the writer made mass collections of *Linaria* about Pensacola and at points inland as far as Atlanta, Georgia. The procedure consisted simply of taking many individuals from each patch of *Linaria*; a number of whole plants were taken and these were supplemented with inflorescences from many additional plants. If a patch had less than fifty individuals, each individual was ordinarily sampled; in very large colonies about fifty individuals were taken at random.

It was found that any patch may consist of one species, or of two species, or of all three species intermixed. Table 1 shows the number of individuals of each species collected in each patch. The stations are arranged in order of distance from the Gulf of Mexico.²

ECOLOGY.—Soil requirements of the three species appear to be identical. About Pensacola, any patch of recently disturbed sand may have any or all species. Anderson (1933) observes that *Iris versicolor* and *I. virginica*, which appear to have entered the

southern peninsula of Michigan at different times, very rarely grow together. There is apparently no such exclusion of one species by another in the colonies of *Linaria*.

Two species sometimes occupy the same habitat, but one is more limited than the other. Such a pair is *Phyllitis Scolopendrium* and *Polystichum Lonchitis* as they occur in Bruce Peninsula, Ontario. Where the *Phyllitis* is found the *Polystichum* is commonly found also, but the converse is not true, for the *Polystichum* is frequently found not accompanied by the *Phyllitis*. It would seem that the occurrence of the *Polystichum* is limited by a certain set of factors, and that of the *Phyllitis* by the same set of factors plus others. The ranges of these two ferns are consistent with this hypothesis, for *Polystichum Lonchitis*, while somewhat local, is widespread in North America (Fernald, 1935, p. 207), but the American phase of *Phyllitis Scolopendrium* is known from but four small areas (Fernald, 1935, p. 200). This case is cited only to serve as a contrast with the *Linaria* situation. *L. floridana* is found, in this part of its restricted range, only along the Gulf of Mexico, and is quite absent from all collections not taken close to the coast. Yet where it does occur it outnumbers the two more widespread and presumably more aggressive species. Why does it fail to spread inland (except for the single inland collection recorded by Pennell, l.c.)? There must be some factor, favorable to *L. floridana* and operative only along the coast (possibly climatic?), to which the other two species are indifferent.

TAXONOMY.—These are three distinct species. All the specimens examined for table 1 had flowers or mature fruits, and most of them had both. There was no question of the specific identity of any individual. And yet *L. canadensis* shows many identical characters, on the one hand with *L. texana*, and on the other with *L. floridana*; in its only unique characters

TABLE 1. Number of individuals collected at each locality.

No.	Location	Miles north of Gulf of Mexico	<i>L.</i> <i>texana</i>	<i>L.</i> <i>canadensis</i>	<i>L.</i> <i>floridana</i>
1	Gulf Breeze Cottages	1.99	3	5	35
2	Gulf Breeze Cottages	2.22	1	45	0
3	Gulf Breeze Post Office	2.56	0	0	58
4	Vacant lot in Pensacola	3.26	5	0	0
5	Fill at north end of Pensacola Bridge	6.36	0	1	53
6	Roadside, Milton, Fla.	17.50	0	12	0
7	Roadside, U. S. 29	18	1	5	0
8	Roadside, U. S. 29	24	0	25	0
9	Roadside, Brewton, Ala.	54	32	4	0
10	14 miles southwest of Atlanta, Ga.	225	0	6	0

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TABLE 2. Characteristics of the three species of *Linaria*.

	<i>L.</i> <i>texana</i>	<i>L.</i> <i>canadensis</i>	<i>L.</i> <i>floridana</i>
Seeds tuberculate, with rounded edges	×
Pedicels glabrous	×
Corolla 10 mm. or more long.....	×
Spur 5-7 mm. long.....	×
Pedice! shorter than the corolla.....	×	×	..
Spur slender	×	×	..
Corolla 7-10 mm. long.....	..	×	..
Spur 2-6 mm. long	×	..
Seeds nearly smooth, with sharp edges.....	..	×	×
Pedicels glandular ^a	×	×
Spur 0.5 mm. long.....	×
Corolla 5-6 mm. long.....	×
Pedice! longer than the corolla.....	×
Spur stout	×

^a *L. canadensis* is commonly described as having glabrous pedicels, but they are glandular on nearly every herbarium sheet examined. In the material of table 1, only the plants of Collection 7 lacked stalked glands, and on these plants the pedicels were so glutinous that grains of sand were adhering to them.

it is intermediate between these two. Table 2 shows the characters of the three species.

On the basis of similarity of flowers, which differ only in size, *L. texana* was at one time reduced (Pennell, 1922, p. 502) to a variety of *L. canadensis* (later to be reinstated as a species by the same author, 1935, p. 302). But, with as sound logic, *L. floridana* and *L. canadensis* might, on the basis of similarity of seeds, be considered as conspecific. Should all three, then, be considered as phases of one polymorphic species? They definitely should not, for in spite of their rather inconspicuous differences and their habit of growing together they remain always perfectly distinct. The 43 individuals in Collection 1, for example, constitute not one variable population, but three distinct populations, which happen to be growing in the same place but among which there is apparently no gene flow.

The only suggestion of hybridization in the group is in Dr. Pennell's report (1935, p. 302) of seeds intermediate in character between *L. canadensis* and *L. texana*, in a belt from Missouri to Texas. Of the nearly 300 individuals examined in table 1, the only one which has any apparent possibility of interchange of characters is in Collection 3 from sandy soil about a recently built house (temporarily serving as the Gulf Breeze Post Office) near the south end of the Pensacola Bridge. This individual, only 12.5 cm. tall, has very small capsules about 1.5 mm. long, and copiously glandular pedicels which are but 3-5 mm. long, much shorter than in normal *L. floridana*. The seeds, while not like those of *L. texana*, have obtuse angles but show to some extent the pebbled surface characteristic of *L. floridana*. There were several hundred individuals in this patch, of which 58 were collected, and all proved to be *L. floridana*.

CONCLUSION.—*Linaria floridana*, *L. canadensis* and *L. texana*, as they occur on the Gulf Coast, meet the definition of species as given by Darlington (1940, p. 150): "the minimum permanently isolated

groups." External isolating factors, such as distribution, habitat preference and flowering time, do not appear to be operative, but internal barriers are sufficient to keep them as three distinct populations.

SUMMARY

Mass collections, consisting of many individual plants or inflorescences from each colony, have been made of the three native species of *Linaria*—*L. floridana*, *L. canadensis* and *L. texana*—from Pensacola, Florida, to north-central Georgia. About Pensacola, where the ranges of the three overlap, they occur together and all flower at the same time. In spite of this fact and the fact that *L. canadensis* is intermediate between the other two, the three species are always perfectly distinct. It is concluded that they represent three populations (therefore, three valid species), which are isolated by internal barriers in lieu of isolation by such external barriers as geographic range, ecology or different flowering seasons.

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PRESENCE OF ANTI-HEMORRHAGIC MATERIAL IN ROOTS OF EUROPEAN BINDWEED (*CONVOLVULUS ARVENSIS* L.)¹

Arthur L. Bakke and Norman D. Render

IN PREPARING roots of the European bindweed (*Convolvulus arvensis* L.) for chemical and physiological studies of root reserves, the root tissue was cut into small sections to facilitate drying. For this purpose, a sharp butcher knife was used, and several times during this process the point of the index finger was accidentally cut. Although the cuts were sufficient for profuse bleeding, bleeding stopped immediately. It was noticed also that the cuts healed readily without any treatment. The experiments submitted in this paper represent an attempt to determine whether a blood-clotting property is present in the roots of the European bindweed.

European bindweed roots were dug, immediately washed and the moisture adhering to the surface removed with the aid of dry cheese cloth. The roots were cut into small pieces, steamed for ten minutes and then placed in a dehydrator (Hixon and Bakke, 1939), where the material was dried under forced air at a temperature which was never above 70°C. When the material was thoroughly dry, it was stored in the dark. Five grams of the powdered root material were placed in a mortar to which 100 cc. of distilled water were added. After mixing thoroughly for ten minutes, the extract was separated from the solid material by filtering through a Büchner funnel. In all cases, the extract was prepared a short time before using. The saline extract was prepared in the same manner except that a physiological salt solution (0.9 per cent) was used instead of distilled water. The solutions were of a chocolate brown color.

In making the blood tests, the patient placed his hand upon the table with palm down. By means of a sharp scalpel provided with a guard so as to penetrate the flesh to a uniform depth, an incision was made in the first joint of the finger. When fewer than four solutions were used, the thumb was not included. Absorbent cotton pads 5×5 cms. were soaked with the extracts and as soon as the incisions were made, were placed directly over the bleeding part. A pledget soaked in distilled water served as a control. Distilled water may possibly have a laking effect on blood. As distilled water and physiological salt solution were considered separately as far as solvents for the bindweed material, the selection of water as a control was entirely logical and valid. At the end of each second, determined by means of a stop watch, the pad was removed and a strip of white blotting paper was immediately placed in contact with the

bleeding part of the finger causing a spot to be formed on the paper. A pad with the extract was again placed on the bleeding part and at the end of one second was again removed to permit the blotting operation. When no spots appeared on the paper on three successive contacts, bleeding was considered to have stopped. When all bleeding had ceased, the spots on each strip of blotting paper were counted. The number of spots on the blotting paper then represented the number of seconds necessary for the completion of bleeding or for the clotting of the blood. If the moistened pledgets became soiled to excess, new pads were used. The data were obtained voluntarily from patients in the psychopathic ward of the Cherokee State Hospital and are presented in the tables.

In tests involving seventeen different individuals (table 1), the saline and water extracts accelerated the blood clotting in all trials, with the exception of patients 4, 13 and 15. The solution used on No. 4

TABLE 1. Blood clotting time as affected by saline and water extracts of European bindweed roots measured by the number of blood spots obtained at one-second intervals upon white blotting paper when placed in contact with the bleeding part (Duke's method).

Designation of patient	Date of test	Blood clotting time in seconds		
		Water	Saline extract	Water extract
1	Oct. 4	37	24	23
2	4	67	37	11
3	11	39	23	16
4*	22	26	44	26
5	23	39	15	21
6	23	25	8	16
7	29	20	10	11
8	29	13	10	11
9	Nov. 8	19	5	10
10	15	56	41	27
11	15	13	12	11
12	15	21	14	14
13	16	18	9	21
14	16	24	15	...
15	16	21	24	8
16	18	23	10	9
17	19	24	11	17

* Solutions one half as strong as those used on other patients.

however accidentally became one half as concentrated as the other extracts. The averages were as follows: physiological salt solution 18, water extract 16, control 28 seconds. In a statistical analysis² of the data, the variation in the response from the different treatments was found to be highly significant.

² Analysis made by the Statistical Laboratory, Iowa State College, Ames, Iowa.

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Norman D. Render, M.D., now superintendent of the Clarinda State Hospital, Clarinda, Iowa, was previously in charge of the clinic at the Cherokee State Hospital, Cherokee, Iowa.

TABLE 2. Blood clotting time as affected by extracts of roots, stems, and leaves of European bindweed and alfalfa leaves determined by the number of blood spots obtained at one-second intervals upon white blotting paper when placed in contact with the bleeding part.

Designation of patient	Date of test	Water	Bindweed leaf and stem water extract	Alfalfa leaf water extract	Bindweed root saline extract
6	Oct. 28	20	7	22	6
7	29	22	16	10	9
8	29	9	9	10	10
9	Nov. 8	9	16	16	7
12	15	21	22	7	9
13	16	24	13	15	16
15	18	28	18	16	18
16	18	16	10	17	17

If this blood-clotting material is present in the roots of the European bindweed, it seemed logical to expect that it might be present in the leaves and stems of this plant, that is in the chlorophyll-bearing tissue. Aqueous extracts were made of the dried leaves and stems of the bindweed. The same control as formerly used was employed, but in addition an aqueous extract of the chlorophyll-bearing parts of the alfalfa plant was introduced as it was readily available and at the same time credited with containing the anti-hemorrhagic vitamin K (Dam, 1929, 1930). The aqueous extract of the alfalfa was of a yellowish green color. On the other hand the aqueous extract of the leaves and stems of the European bindweed was of a dark brown color somewhat darker than the root extract. The results of the eight series of determinations involving the three different plant tissues along with the control are presented in table 2.

The data in table 2 show that the extracts of the bindweed roots and the chlorophyll-bearing parts have a retarding effect on bleeding time in humans. The saline extract of the root tissue of the European bindweed generally gave lower blood-clotting values than was present for the water extract of the leaf and stem tissue of the same plant. Of course it is realized that the evidence was drawn from only eight cases. In a statistical analysis of these data, the degree of variance in the response from the different extracts was significant.

In examining the data further, it is clear that there is present some constituent (or constituents) in the roots of the European bindweed which accelerates

the clotting of human blood. The material is water soluble or soluble in a weak salt solution. Being water soluble, it cannot be placed directly in the vitamin K category (Smith *et al.*, 1939). Whether the European bindweed material will act in the same way as young chlorophyll-bearing parts of alfalfa or grasses or phythiocol must await experimental evidence. As the European bindweed is plentiful and readily obtained, it may find a place in the production of valuable therapeutic compounds.

SUMMARY

The results of this study show that there is present in the European bindweed (*Convolvulus arvensis* L.) a substance (or substances) which causes the clotting of human blood.

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A CYTOLOGICAL STUDY OF SOME SPECIES IN THE TRIBE PANICEAE¹

Glenn W. Burton

Most of the grasses now included in improved pastures in the Southeast belong to the tribe Paniceae. Many of the native grasses in this region are likewise members of this large tribe, which is confined primarily to the tropical or semi-tropical portion of the world.

Comparatively few species in the Paniceae have been studied cytologically. The chromosome counts that have been reported for members of this tribe have been assembled in table 1. The need of more cytological information on these grasses, some of which are included in the grass breeding activities at the Georgia Coastal Plain Experiment Station, Tifton, Georgia, motivated the cytological investigations reported here.

MATERIALS AND METHODS.—Most of the *Panicum* species and several of the other grasses mentioned in this study were obtained from Mr. George E. Ritchey at the Florida Agricultural Experiment Station, Gainesville, Florida. They were collected as promising specimens in some of the native pastures in Florida and were planted for observation in Ritchey's introduction garden. They were identified with the aid of the Manual of Grasses of the United States (Hitchcock, 1935) and identifications were checked by Mr. J. R. Swallen, U. S. Department of Agriculture, Washington, D. C. Vegetative material of these plants was grown in the greenhouse at Tifton, Georgia, for root tip production.

Root tips from all of the grasses studied in this report were collected between 8 and 10 A.M. and were fixed in Craf fixative for twenty-four hours. The embedding procedure consisted of a slight modification of the alcohol-chloroform method as revised by LaCour (1937). Sections were cut 10 μ thick and were stained by the iodine-gentian violet technique described by LaCour. All chromosome numbers were determined by making camera lucida drawings of several different equatorial plates in each species. The drawings were made at table level using a 1.5 mm. objective and a 25 \times ocular, and all have the same degree of magnification.

OBSERVATIONS.—A list of the Paniceae examined, together with their somatic chromosome numbers and their source, is presented in table 2. Herbarium specimens have been made of most of these grasses, and these have been deposited either in the herbarium of the University of Florida at Gainesville or in that of the Coastal Plain Experiment Station,

TABLE 1. Somatic chromosome numbers of several of the Paniceae genera as reported in the literature.

Species	Somatic chromosome number (2n)	Reference
<i>Panicum anceps</i> Michx.	18	(11) ^a
<i>P. capillare</i> L.	18	(1)
<i>P. dichotomiflorum</i> Michx.	54	(5)
<i>P. lindheimeri</i> Nash	18	(5), (11)
<i>P. miliaceum</i> L.	42	(12)
<i>P. miliaceum</i> L.	36	(2)
<i>P. miliare</i> Lam.	36	(12)
<i>P. scribnerianum</i> Nash	18	(5)
<i>P. sphaerocarpon</i> Ell.	18	(5)
<i>P. subvillosum</i> Ashe	18	(5)
<i>P. texanum</i> Buckl.	36	(11)
<i>P. tsugetorum</i> Nash	18	(5)
<i>Paspalum boscianum</i> Flugge	40	(4)
<i>P. ciliatifolium</i> Michx.	20	(4)
<i>P. dilatatum</i> Poir.	40	(10), (4)
<i>P. floridanum</i> Michx.	160	(4)
<i>P. malacophyllum</i> Trin. F. C. 04240	40	(4)
<i>P. notatum</i> Flugge	40	(4)
<i>P. notatum</i> P. I. 121415	40	(4)
<i>P. paniculatum</i> L. P. I. 128190....	20	(4)
<i>P. pubescens</i> Muhl.	20	(5)
<i>P. scrobiculatum</i> L.	40	(1)
<i>P. stoloniferum</i> Bosc	20, 20-23	(10), (3)
<i>P. urvillei</i> Steud.	40	(11), (4)
<i>P. urvillei</i> Steud.	60	(11)
<i>P. virgatum</i> L.	80	(2)
<i>Pennisetum cenchroides</i> (L.) Rich.	36	(2)
<i>P. clandestinum</i> Hochst. ex Chiov.	36	(9)
<i>P. glaucum</i> (L.) R. Br.	14	(8), (12)
<i>P. longistylum</i> Hochst.	45	(2)
<i>P. macrourum</i> Trin.	54	(2)
<i>P. orientale</i> L. Rich.	36	(1)
<i>P. ruppelii</i> Steud.	27	(2)
<i>P. setosum</i> (Swartz) L. Rich.	54	(1)
<i>P. villosum</i> R. Br.	45	(2)
<i>Digitaria exilis</i> (Kipp.) Stapf....	54	(7)
<i>D. horizontalis</i> Willd.	36	(1)
<i>D. sanguinalis</i> (L.) Scop.	28	(5)
<i>D. sanguinalis</i> (reported as <i>Panicum sanguinale</i> L.)	36	(1)

^a Numbers in parenthesis refer to literature cited list.

Tifton, Georgia. A brief discussion of each species follows.

Panicum antidotale Retz. F. C. 22396.—This rather tall, stemmy species, obtained from Mr. S. A. Hagler, Fulton, Kentucky, has been quite susceptible to disease at Tifton, Georgia, and has grown well only on soils of unusually high fertility. Since the data presented in tables 1 and 2 indicate that 9 is the most common basic chromosome number in this genus, the plants examined here may be considered diploid, 2n=18 (fig. 1).

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1



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TABLE 2. Somatic chromosome numbers in the Paniceae examined at Tifton, Ga.

Text figure no.	Species and number	Seed or clone source	Somatic chromosome number (2n)
1	<i>Panicum antidotale</i> F. C. 22396	Fulton, Ky.	18
2	<i>P. arenicoloides</i> 97N	Gainesville, Fla.	18
3	<i>P. commutatum</i> 959N	Gainesville, Fla.	18
4	<i>P. anceps</i> 449	Gainesville, Fla.	36
5	<i>P. sp.</i> P. I. 126491	Sydney, N. S. W., Australia	36
6	<i>P. purpurascens</i>	Tifton, Ga.	36
7	<i>P. fasciculatum</i>	Lambert Seed Co., Darlington, Ala.	36
8	<i>P. paludivagum</i> 302N	Gainesville, Fla.	36
9	<i>P. texanum</i>	Auburn, Ala.	54
10	<i>P. jubiflorum</i> P. I. 126489	Sydney, N. S. W., Australia	54
11	<i>P. virgatum</i> 490N	Gainesville, Fla.	72
12	<i>Paspalum supinum</i> 319N	Gainesville, Fla.	20
13	<i>P. quadrifarium</i> F. C. 04217	Buenos Aires, Argentina	30
14	<i>P. intermedium</i> F. C. 04235	Buenos Aires, Argentina	40
15	<i>P. langei</i> 677N	Gulf Hammock, Fla.	60
16	<i>P. longipilium</i> 926N	Shamrock, Fla.	60
17	<i>P. giganteum</i> 730N	Gulf Hammock, Fla.	120
18	<i>P. alnum</i>	College Station, Tex.	24
19	<i>P. distichum</i>	Micanopy, Fla.	48
20	<i>Pennisetum glaucum</i> (commercial)	Tifton, Ga.	14
21	<i>P. glaucum</i> (Russian)	Leningrad, Russia	14
22	<i>P. purpureum</i>	Tifton, Ga.	28
23	<i>Digitaria pentzii</i> P. I. 77998	South Africa	36
24	<i>D. polezansii</i> P. I. 111105	South Africa	34
25	<i>D. decumbens</i> P. I. 111109	South Africa	30
26	<i>D. sp.</i> P. I. 111128	South Africa	36
27	<i>Axonopus affinis</i>	Tifton, Ga.	80

Panicum arenicoloides Ashe.—This perennial bunch grass, which is adapted to the sandy pine woods of the Southeast, has 18 somatic chromosomes (fig. 2).

Panicum commutatum Schult.—Although found in most of the states east of the Mississippi River, this perennial grass is of limited economic importance. The specimen examined showed the 2n number to be 18 (fig. 3).

Panicum anceps Michx.—This rather large, rhizomatous perennial has been found growing in moist sandy soils from New Jersey to Kansas and south to the Gulf of Mexico. Since Nielsen (1939) reported 18 somatic chromosomes in a plant of this species collected in Rosston, Arkansas, the count of 36 here obtained for the Florida specimen demonstrates the existence of intraspecific chromosome races in this species (fig. 4).

Panicum sp. L. P. I. 126491.—Mr. Swallen reports that this introduction from Australia does not fit descriptions in the manuals and, hence, is still unidentified. The material examined was tetraploid, 2n=36 (fig. 5).

Panicum purpurascens Raddi.—Paragrass, a stoloniferous, robust perennial, is palatable, nutritious, and highly productive. In tropical America it is one of the best pasture grasses, supporting several mature cattle per acre under favorable conditions. Its cold susceptibility restricts it largely to the State of Florida. It was found to have 36 somatic chromosomes (fig. 6).

Panicum fasciculatum Swartz.—This vigorous annual, commonly called brown top millet, is cultivated in the Southeast for its seed and forage. Root tip cells contained 36 chromosomes (fig. 7).

Fig. 1-27. Camera lucida drawings of chromosomes on equatorial plates in cells from root tips of the following Paniceae.—Fig. 1. *Panicum antidotale* F. C. 22396, 2n=18.—Fig. 2. *P. arenicoloides* 97N, 2n=18.—Fig. 3. *P. commutatum* 959N, 2n=18.—Fig. 4. *P. anceps* 449, 2n=36.—Fig. 5. *P. sp.* P. I. 126491, 2n=36.—Fig. 6. *P. purpurascens*, 2n=36.—Fig. 7. *P. fasciculatum*, 2n=36.—Fig. 8. *P. paludivagum* 302N, 2n=36.—Fig. 9. *P. texanum*, 2n=54.—Fig. 10. *P. jubiflorum* P. I. 126489, 2n=54.—Fig. 11. *P. virgatum* 490N, 2n=72.—Fig. 12. *Paspalum supinum* 319N, 2n=20.—Fig. 13. *P. quadrifarium* F. C. 04217, 2n=30.—Fig. 14. *P. intermedium* F. C. 04235, 2n=40.—Fig. 15. *P. langei* 677N, 2n=60.—Fig. 16. *P. longipilium* 926N, 2n=60.—Fig. 17. *P. giganteum* 730N, 2n=120.—Fig. 18. *P. alnum*, 2n=24.—Fig. 19. *P. distichum*, 2n=48.—Fig. 20. *Pennisetum glaucum* (commercial), 2n=14.—Fig. 21. *P. glaucum* (Russian), 2n=14.—Fig. 22. *P. purpureum*, 2n=28.—Fig. 23. *Digitaria pentzii* P. I. 77998, 2n=36.—Fig. 24. *D. polezansii* P. I. 111105, 2n=34.—Fig. 25. *D. decumbens* P. I. 111109, 2n=30.—Fig. 26. *D. sp.* P. I. 111128, 2n=36.—Fig. 27. *Axonopus affinis*, 2n=80. Magnification of all figures ca. 2,000X.

Panicum paludivagum Hitch. and Chase.—This creeping perennial is found more or less submerged in fresh water rivers and lakes in Florida, Texas, and Mexico. The material examined was tetraploid, $2n=36$ (fig. 8).

Panicum texanum Buckl.—Seed of this cultivated annual, Texas millet, supplied by Dr. D. G. Sturkie, Auburn, Alabama, gave rise to hexaploid seedlings, $2n=54$. Nielsen's (1939) count of 36 demonstrates the existence of intraspecific chromosome races in this species (fig. 9).

Panicum jubiflorum Trin. P. I. 126489.—This perennial introduction from Australia possessed 54 somatic chromosomes (fig. 10).

Panicum virgatum L.—Switchgrass, a vigorous, rhizomatous perennial, ranging from three to six feet in height when mature, is widely distributed in the prairies of the United States. The robust Florida specimen studied in this instance was octoploid, $2n=72$ (fig. 11).

Paspalum supinum Bosc.—This small perennial grows in the dry sandy open ground in the Coastal Plain from North Carolina to Louisiana. It showed 20 chromosomes in its root tips (fig. 12).

Paspalum quadrifarium Lam. F. C. 04217.—Introduced from Argentina, this robust perennial bunch grass has been one of the most frost and cold tolerant *Paspalums* grown at Tifton, Georgia. Its very poor seeding habit and lack of palatability, however, materially limit its economic value. All material examined possessed 30 somatic chromosomes (fig. 13). The high degree of sterility of *P. quadrifarium* and the fact that no species with less than 20 somatic chromosomes has been found suggest that it may have arisen recently as a hybrid between diploid and tetraploid races or species.

Paspalum intermedium Munro. F. C. 04235.—This vigorous perennial bunch grass introduced from Argentina had 40 somatic chromosomes (fig. 14).

Paspalum langei (Fourn.) Nash.—This perennial bunch grass that occurs in moist woods in Florida and near the Gulf of Mexico was found to be hexaploid, $2n=60$ (fig. 15).

Paspalum longipilum Nash.—The Florida specimen of this perennial bunch grass, which has been found in the damp sandy soils of the Coastal Plain from New Jersey to Texas, had 60 somatic chromosomes (fig. 16).

Paspalum giganteum Baldw.—This robust perennial, with short scaly rhizomes and a height of four to six feet when mature, is limited to the lower Coastal Plain of the Gulf States. The plant collected in Florida and examined in this study was duodecaploid, $2n=120$ (fig. 17).

Paspalum alnum Chase.—Commonly called Coombs *Paspalum*, this rather small tufted perennial grows in South America and Jefferson County, Texas, where it is considered an excellent pasture species. At Tifton, Georgia, however, it has failed to survive the winter. Seedling plants of this grass obtained from Texas possessed 24 somatic chromosomes that were twice the size of the other *Paspalum*

chromosomes studied (fig. 18). Thus a new basic number of either 6 or 12 for the *Paspalum* genus is suggested.

Paspalum distichum L.—Knotgrass is a widely distributed stoloniferous species that has been found growing in moist, usually warm, places of both hemispheres. A study of a large number of equatorial plates of this grass left no doubt but that the material examined possessed 48 chromosomes as the $2n$ number (fig. 19). This furnishes further evidence of the existence of a basic number of 6 or 12 in addition to the common number of 10.

Pennisetum glaucum (L.) R. Br.—Cultivated since prehistoric times in Africa and Asia for forage and human food, cat-tail or pearl millet has become a most important temporary pasture crop for cattle and hogs in several of the southeastern states. Seed to plant these temporary pastures has been produced in the Southeast for many years, yet no strains or varieties of this grass are on the market. Plants from this commercial seed usually reach a height of five to eight feet when mature and are about as variable as individuals in a commercial open pollinated variety of corn. Fourteen very large chromosomes were found in the root tip cells of a number of different seedlings of this grass (fig. 20).

Pennisetum glaucum (L.) R. Br., Russian.—In 1937 the U. S. Department of Agriculture obtained from Leningrad, Russia, several lots of seed of cat-tail millet. This seed carrying the P. I. numbers 115055 to 115059, inclusive, gave rise to plants that reached a mature height of eight to twelve feet, possessed very woody stems, were extremely heterozygous and were so late that very few matured seed the first year. A number of selections from this material are taller, woodier, later maturing and produce more leaves per stem than commercial cat-tail millet. They also seed less freely than the commercial strain. A cytological study of root tips of several of these strains revealed that they possessed 14 somatic chromosomes which seemed identical in their gross morphology with those of the smaller common cat-tail millet (fig. 21).

Pennisetum purpureum Schumach.—Introduced from Africa in 1913, this coarse perennial, commonly called napiergrass, resembles sugarcane vegetatively and reaches a height of eight to sixteen feet when mature. It has been found to be an excellent pasture plant when managed properly in a deferred system of grazing. Its cold susceptibility has restricted it to Florida and the lower Coastal Plain of the Gulf States. Several different introductions (among them P. I. 52182) and selections of this grass possessed 28 chromosomes as the $2n$ number (fig. 22).

Digitaria pentzii Stent. P. I. 77998.—Were it not for its poor seeding habits, this stoloniferous South African perennial would be a valuable pasture grass in much of the Southeast. It showed 36 somatic chromosomes (fig. 23).

Digitaria polevansii Stent. P. I. 111105.—A study of a large number of somatic complements in this

African introduction revealed 34 chromosomes as the $2n$ number (fig. 24).

Digitaria decumbens Stent. P. I. 111109.—This African introduction spreads rapidly by long prostrate stolons and does not form the tufted bunches characteristic of *D. pentzii*. It is more susceptible to winter killing than most of the *D. pentzii* introductions. Thirty chromosomes were found in the root tip cells (fig. 25).

Digitaria sp. P. I. 111128.—This South African introduction differs from the other three mentioned above by having short rhizomes, no above-ground stolons, and relatively few basal leaves. Mr. Swallen states that he has been unable to find a published description of this grass. It had 36 somatic chromosomes (fig. 26).

Axonopus affinis Chase.—The excellent seeding habits, ability to grow at low fertility levels, stoloniferous habit and tolerance of close grazing are features of this perennial which have made it, carpet-grass, well adapted to the moist sandy soils of the Coastal Plain. These characteristics are also responsible for its widespread use in improved pasture mixtures and its natural spread in many native pastures. Examination of a number of equatorial plates in the root tips of this species showed it to have 80 chromosomes (fig. 27).

SUMMARY

The somatic chromosome numbers in 27 races belonging to 26 species of the tribe Paniceae are presented.

The eleven *Panicum* species examined in this study proved to be regular members of a polyploid series having 9 as the basic number. Races of *P. anceps* and *P. texanum* were found that were higher in the polyploid series than those previously reported, demonstrating the existence of intraspecific polyploidy in those species.

Five of the eight *Paspalum* species studied occupied diploid, tetraploid, hexaploid and duodecaploid positions in a series having 10 as the basic number and extending to the hexadecaploid *P. floridanum* Michx. (1940). It is suggested that the highly sterile *P. quadrifarium*, having only 30 somatic chromosomes, arose as a hybrid between diploid and tetraploid races or species. *P. alnum* and *P. distichum*, two closely related species with 24 and 48 somatic chromosomes, respectively, demonstrate the existence of a new basic number of either 6 or 12 in the genus *Paspalum*.

Eight of the nine *Pennisetum* species reported in the literature fit a polyploid series having 9 as the

basic number. Numerous specimens of *P. glaucum* and *P. purpureum* possessed 14 and 28 chromosomes, respectively, indicating that 7 is also a basic number in this genus.

Four introductions of the highly sterile perennial *Digitaria* species from South Africa gave the irregular counts of 30, 34, 36 and 36 somatic chromosomes suggesting that some of them may be of recent hybrid origin.

Axonopus affinis collected at Tifton, Georgia, contained 80 somatic chromosomes.

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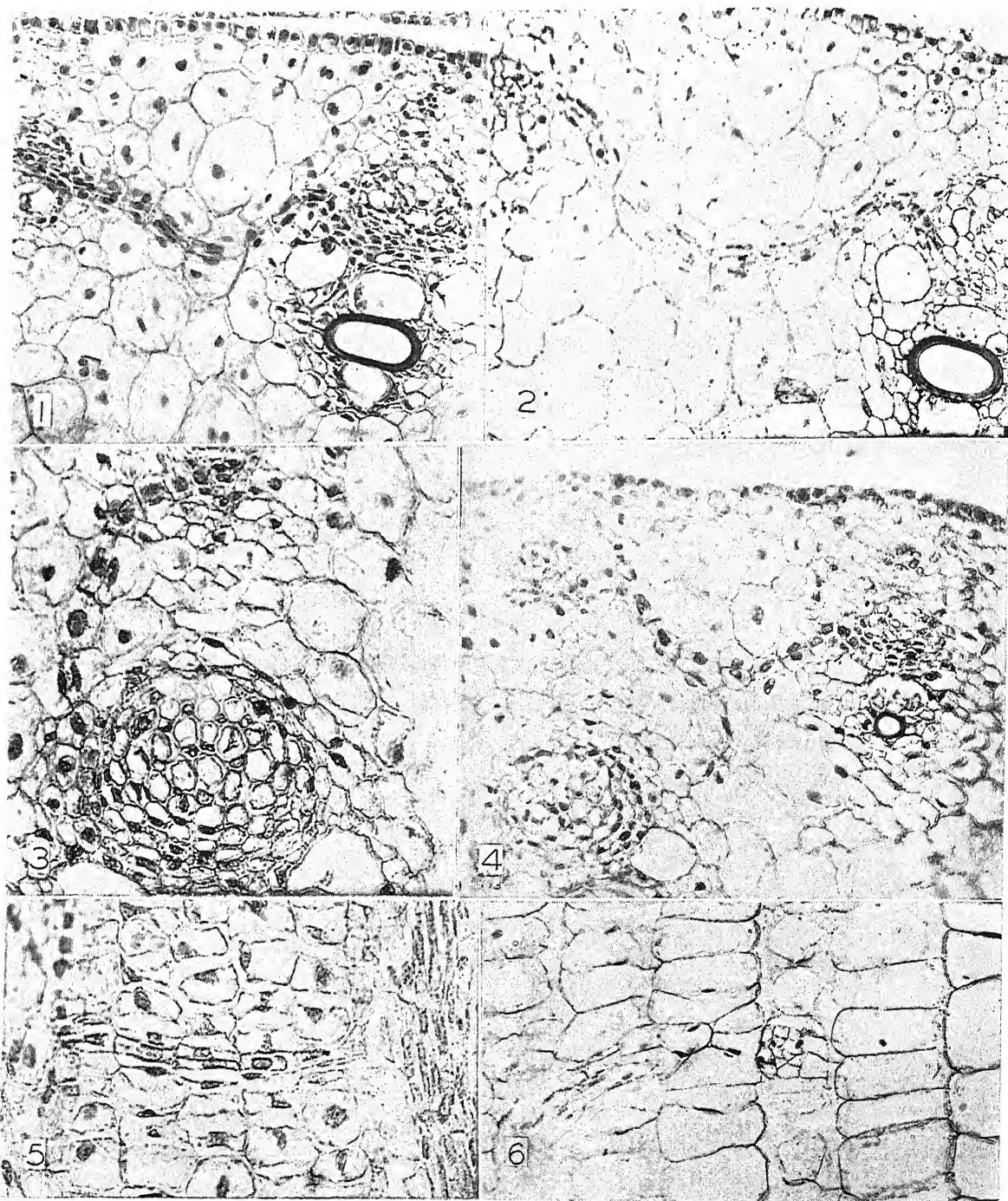


Fig. 1-6.—Fig. 1. Cross section of leaf-sheath showing early stage in the development of a cross-connecting veinlet. $\times 238$.—Fig. 2. Cross section of leaf-sheath showing “interfascicular” cambium connecting with intrafascicular cambium of a vertical bundle. $\times 238$.—Fig. 3. Cross section of leaf-sheath showing connection developing between bundles in a radial direction. $\times 400$.—Fig. 4. Cross section of leaf-sheath showing branching in developing veinlet. $\times 238$.—Fig. 5. Tangential section of leaf sheath showing early stage in the development of cross-connecting vascular tissue. $\times 400$.—Fig. 6. Radial section of leaf-sheath showing cross section of a young veinlet. $\times 238$.

THE DEVELOPMENT OF VASCULAR CONNECTIONS IN THE LEAF-SHEATH OF SUGARCANE¹

Chas. F. Moreland and Lewis H. Flint

ONE OF the characteristics of monocotyledonous plants as distinguished from dicotyledonous plants is the absence of cambium in the vascular bundle, all development arising through the differentiation of primary tissues. However, Arber (1918) has drawn attention to the rather widespread occurrence of intrafascicular cambium in this group and Artschwager (1925) called attention to cambium-like cells between the xylem and phloem of the bundles of the leaf-sheath of sugarcane. Our observations revealed vestigial cambial activity in the bundles of the leaf-sheath of sugarcane and in addition the development of an interfascicular cambium-like meristem which gave rise to cross-connecting veinlets.

Arber (1925) described cross-vascular connections between the parallel veins of the leaves of several monocotyledons. Avery (1930) described similar structures for corn, and Artschwager (1925) noted connections of the same type in sugarcane. In describing these cross-connecting veinlets none of the mentioned investigators described the way in which these connections originated.

In order to make a study of the mode of origin of the cross-veinlets, segments were obtained from the spindle of the sugarcane stalk (variety C. P. 29-320). Since the spindle consists of concentric layers of leaf-blades and leaf-sheaths of various ages surrounding the terminal bud of the stalk, segments from this region contained tissues in various stages of development. The segments were imbedded in paraffin for microtome sectioning, and sections were cut in three different planes—transverse, radial, and tangential.

Transverse sections of the spindle segments proved to be best for determining the origin of cross-connections between the vertical bundles of the sheath. Young leaf-sheaths could be found in which the primary vascular tissues were well differentiated. The protoxylem elements and the sieve tubes and companion cells of the phloem were fully developed. The metaxylem cells were in the process of maturation. Between the xylem and phloem of many of the bundles could be seen a cambium-like zone, that is, small rectangular cells arranged in radial rows (fig. 2). The parenchymatous cells between the vascular bundles were quite large and loosely arranged, with intercellular spaces between them. Occasionally within the parenchyma tissue it was noted that by a seemingly concerted activity a continuous row or chain of more or less mature parenchyma cells extending from one of the vascular bundles of the vertical system to another became meristematic and underwent an almost simultaneous internal division. This division took place in such a way that the origin of an interfascicular cambium or a cork cambium was simulated. Photomicrographs of early stages of de-

velopment are given in figures 1 and 2. It was further observed that the intervening meristematic cells connected in a tangential direction with the intrafascicular cambium of the vertical bundles involved. Thus the early stages in the development of the cross-connecting vascular tissue further suggested an interfascicular cambium (fig. 2). Of course the analogy cannot be carried very far because the interfascicular cambium of dicotyledonous stems is continuous in a vertical plane and gives rise to conducting cells of the vertical system.

The connecting veinlets were not only found connecting bundles in a tangential direction but were also observed connecting bundles in a radial direction (fig. 3). In some cases they were found to branch and connect bundles in a radial and tangential direction at the same time; that is, three bundles of the vertical system were involved (fig. 4). The branching was noted to have arisen in one original parenchyma cell. The first division of this parenchyma initial resulted in the formation of two sets of cells. Each set had its distinctive alignment.

The origin of the cross-connection veinlets was interpreted to have been of a secondary nature in that they arose from living cells that had become permanent and then returned to the meristematic condition. After the formation of the initials had taken place, the divisions which followed were in two planes at right angles to each other and parallel with the row of initials. The first two divisions appeared to be about simultaneous. Thus observations showed an initial first divided into four cells, two cells in a horizontal and two in a vertical plane (fig. 3 and 5). This was quickly followed by other divisions which resulted in the formation of about four rows of cells in each of the two planes. Thus the veinlets were about square as seen in cross section (fig. 6). The long axes of the young cells were parallel with the row of initials and their lengths were the same as the diameters of the mother cells from which they had been derived. Each group of derivatives was within the area formerly occupied by the mother cell. This regular arrangement and mode of origin indicated that the cross-connecting veinlets were composed of secondary vascular tissues.

SUMMARY

Radial and tangential cross-veinlets connecting vertical vascular bundles were observed within the leaf-sheaths of sugarcane. These cross-veinlets were distributed at approximately regular intervals in the vertical plane and were occasionally branched.

The cross veinlets originated within a single or branched horizontal line of parenchyma cells and for the most part remained restricted within the old boundaries of these cells. Temporary meristem was continuous with similar meristem within the vertical

¹ Received for publication February 2, 1942.

bundles, and differentiation extended to the elaboration of xylem and phloem.

The development of a secondary meristem and the extension of such cambium from one vertical bundle to another indicated activities within monocotyledonous plants similar to some of those more commonly associated with dicotyledonous plants.

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TOMATO RINGSPOT ON CURRANT¹

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THE TRANSMISSION of virus diseases from woody to herbaceous plants and vice versa has been uniformly unsuccessful, according to published reports. In a borderline case, however, Smith (1937a) found that a red currant (*Ribes* sp.) developed numerous small "water-clear" lesions on the youngest leaves when inoculated by rubbing with tobacco necrosis virus. This observation was confirmed by the writer on *Ribes sativum*, and interestingly enough, aside from the initial lesions, no further pathological developments followed.

Two distinct virus diseases are now known to occur on the red currant in, respectively, the northeastern United States (Hildebrand, 1939) and Germany (Winter, 1940). Efforts at transferring the American currant mosaic disease from plant to plant by mechanical methods have always been unsuccessful.

At the Rockefeller Institute in 1939-40, during an investigation aimed at the discovery of the insect vector of currant mosaic, diseased currant plants were grown under different temperature conditions to study the effect on the insects harbored by the plants. Typical currant mosaic symptoms (fig. 1) developed only on the plants incubated at the lowest temperature range (60°-70°F.). The plants held at the higher temperatures failed to develop chlorosis either in the greenhouse during the winter or spring or after transplanting outdoors for the remainder of the 1940 season. Proof that the virus symptoms had been masked by high temperatures was obtained when the plants under consideration again exhibited typical chlorosis in the outdoor planting in 1941.

In early February, 1940, during one of the frequent examinations made of the plants which failed to develop chlorosis, peculiar chlorotic ring patterns (fig. 2) were noted on occasional young leaves in the 70°-80°F. greenhouse. The zonate chlorotic spots which ordinarily failed to persist when the leaves matured had not been seen hitherto on the currant and suggested the presence of another virus disease

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This study was initiated and the greater portion of it carried out in the Plant Pathology Division of the Rockefeller Institute for Medical Research at Princeton, N. J., while the writer held a John Simon Guggenheim Memorial Foundation Fellowship during 1939-1940.

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in addition to currant mosaic. Because of their shape the spots aroused suspicion as to a possible relationship to several well known ringspot-virus diseases and invited an attempt to transmit the disease by mechanical methods.

The present report is concerned with this new virus disease of currant, its transmission by mechanical means to tobacco, and its identification as a strain of tomato ringspot virus (*Annulus zonatus* H., Holmes, 1939). Studies on its possible relationship to the mosaic disease with which it was associated in currant have not been concluded.

TRANSMISSION EXPERIMENTS FROM CURRANT TO TOBACCO.—The first transmission experiment conducted in February, 1940, when occasional leaves of the new growth on currant were beginning to show distinctive ringspot symptoms, gave rise to zonate necrotic lesions on tobacco, proving that a sap transmissible virus was present in the currant. Two or more plants of each of the following species—*Nicotiana tabacum* L. var. Turkish, *N. rustica* L., *N. glutinosa* L., *N. langsdorffii* Weinm., *N. sylvestris* Speg. and Comes, China aster (*Callistephus chinensis* Nees.), cowpea (*Vigna sinensis* (L.) Endl. vars. Black and Blackeye), bean (*Phaseolus vulgaris* L. var. Early Golden Cluster), Wilder red currant (*Ribes sativum* L.), and tomato (*Lycopersicon esculentum* Mill. var. Bonny Best)—were inoculated by the rubbing method with fresh juice extract, in all cases at least one plant being dusted with carborundum beforehand. On the seventh day, three ringspot lesions were observed on a Turkish tobacco plant and twenty-one and twelve lesions, respectively, on two *N. rustica* plants all of which had received the carborundum treatment. About twenty of the lesions were removed with a cork borer and used for sub-inoculating *N. rustica*, *N. glutinosa*, China aster, and Turkish tobacco, one lesion to the plant. Abundant ringspot lesions appeared in all inoculated plants of Turkish tobacco and *N. rustica*. In one out of two cases infection resulted on aster. Although distinct symptoms did not develop on plants of *N. glutinosa*, transmission experiments demonstrated that they contained the virus.

Returning to the masked mosaic diseased currants at intervals of about one week, seven successive trials

were attempted from the original and subsequent consignments of plants brought indoors from the Hudson Valley source, with similar positive results in six cases. All six isolation experiments with leaves from healthy plants obtained from a New York nursery gave negative results. The fact that juice extract from mosaic diseased currants from two other sources (New York and New Jersey) in six different trials failed to induce the ringspot disease on tobacco indicated rather definitely that, since the ringspot disease was not always associated with the mosaic disease, it was in all probability a chance contaminant and a distinct new disease of the currant.

TRANSMISSION EXPERIMENTS FROM TOBACCO TO CURRANT.²—Transmission of the ringspot virus from tobacco back to currant by mechanical means has not been accomplished thus far. The virus content of the expressed juice from infected tobacco plants which was employed was between 100 and 1,000 times as great as that of juice from currant. Various devices such as rubbing, needle-pricking and injection seemed to be equally ineffective. Such variations in methods for inoculating currant plants as rubbing with and without carborundum, rubbing the upper and lower leaf surfaces, inoculating different-aged leaves, the use of juice diluted and nondiluted, and of juice with and without buffer, inoculation at different hours of the day, employing several types of needle puncture, injection into the stems with a syringe, and the use of plants of different ages and sizes, all proved ineffective for reproducing the ringspot symptoms. Details of these mechanical transmission tests, which involved forty experiments and over 200 currant plants have been omitted because the results have always been negative.

IDENTIFICATION OF CURRANT RINGSPOT VIRUS.—*Transmission experiments.*—Sap transmission from tobacco to tobacco and from the various susceptible plants back to tobacco was readily accomplished by the rubbing technique. The number of lesions was increased about ten-fold by first dusting with carborundum powder. The zonate necrotic spots on Turkish tobacco were similar in appearance to those produced by tomato-ringspot virus (Price, 1936; Holmes, 1939); similar to, but distinct from, those produced by tobacco-ringspot virus (Price, 1936); and distinct from those caused by the tomato-ringspot virus described by Imle and Samson (1937). The spots had begun to appear by the second day

² One of the problems encountered in these studies was that of securing suitable small currant plants for transmission experiments. Cuttings taken in the fall produced roots and a few leaves but could not be used because they proved to be partially dormant. Cuttings started in March, 1940, rooted readily and produced excellent plants. The commercial rooting stimulant, Hormodin No. 1 powder, proved effective for speeding up root production. Three weeks from the time the cuttings were made slightly over 90 per cent of those which received the stimulant as compared to about 9 per cent of the untreated ones were well rooted and ready for potting. The untreated cuttings required about twice as long for good rooting. The same procedure was used again in the 1941 studies but with less striking results from the rooting stimulant.

following inoculation. Similar systemic lesions appeared later. Upon reaching the systemic stage the subsequent plant growth was symptomless. Such plants had apparently developed what has been called specific, non-sterile immunity and were found not susceptible to reinfection.

Influence of buffers on infectivity.—Mixing infective juice extract from tobacco with a buffer solution (0.1 M. phosphate buffer, pH 7.0) failed to increase infectivity. For example, in one experiment at a dilution of 1:10 the average number of lesions per plant with buffer was 46 as against 71 lesions where distilled water was used. In another experiment the two treatments gave practically identical results of 48 and 56 lesions.

Influence of age of plant on susceptibility.—In general, the very young plants were much more susceptible to infection than the older ones. The currant ringspot virus was found to be of relatively high concentration in young plants of nearly all species found susceptible to infection. Young *Nicotiana rustica* plants were much more susceptible than older ones, and as many as 50 per cent died in some tests. With pokeweed, age of plant seemed to make little difference in susceptibility. Pokeweed and cowpea plants largely failed to survive infection. Only tiny tomato plants between two and three inches tall were readily inoculated. The appearance of concentric zonate necrotic spots was followed by extreme stunting (fig. 4), abnormal string-like growth of the leaves, and failure to bear fruit. While the virus became systemic in several of the susceptibles. Turkish tobacco was employed as test plant in the immunization studies discussed later.

HOST RANGE.—The host range of the currant-ringspot virus, as far as it has been determined, closely agrees with that of tomato ringspot (Price, 1940a). The following plants, taken at random from the list tested by Price, have been found susceptible, in all cases the virus being reisolated from the inoculated plants: *Amaranthaceae*—*Amaranthus caudatus* L.; *Apocynaceae*—*Vinca rosea* L.; *Caryophyllaceae*—*Lychnis chalconica* L.; *Chenopodiaceae*—*Beta vulgaris* L.; *Compositae*—*Callistephus chinensis* Nees., *Helianthus annuus* L., *Zinnia elegans* Jacq.; *Crassulaceae*—*Sedum acre* L.; *Cruciferae*—*Lobularia maritima* Desv.; *Cucurbitaceae*—*Cucumis sativus* L.; *Euphorbiaceae*—*Ricinus communis* L.; *Geraniaceae*—*Pelargonium hortorum* Bailey; *Gesneriaceae*—*Sinningia speciosa* Benth. and Hook.; *Hydrophyllaceae*—*Nemophila insignis* Benth.; *Labiatae*—*Coleus blumei* Benth.; *Leguminosae*—*Phaseolus vulgaris* L. var. Early Golden Cluster, *Vigna sinensis* Endl.; *Phytolaccaceae*—*Phytolacca decandra* L.; *Polemonaceae*—*Phlox drummondii* Hook.; *Resedaceae*—*Reseda odorata* L.; *Saxifragaceae*—*Ribes sativum* Syme. var. Wilder; *Scrophulariaceae*—*Cymbalaria muralis* Gaertn., Mey. and Scherb.; *Solanaceae*—*Lycopersicon esculentum* Mill. var. Bonny Best, *Nicotiana glutinosa* L., *N. langsdorffii* Weinm., *N. rustica* L., *N. sylvestris*

Speg. and Comes, *N. tabacum* L. var. Turkish, and *Solanum nodiflorum* L.

CROSS IMMUNIZATION.—Cross-immunization experiments with two other ringspot viruses have resulted in protection only against tomato ringspot, suggesting a close relationship to this virus. The mechanism of acquired immunity in plants and the procedure for obtaining plants for cross-immunization studies have been discussed in detail by Price (1940c). Briefly, the procedure depends on the fact

that small inoculated plants recover from the disease symptoms while still containing the virus. Cuttings made from these plants and rooted in a mixture of peat and sand were ready for use in about six weeks from the time the inoculations were made and, except for a slight loss in vigor, were normal in appearance.

When inoculated with the currant ringspot virus, typical symptoms were produced on the plants immunized against tobacco ringspot, on healthy seed-

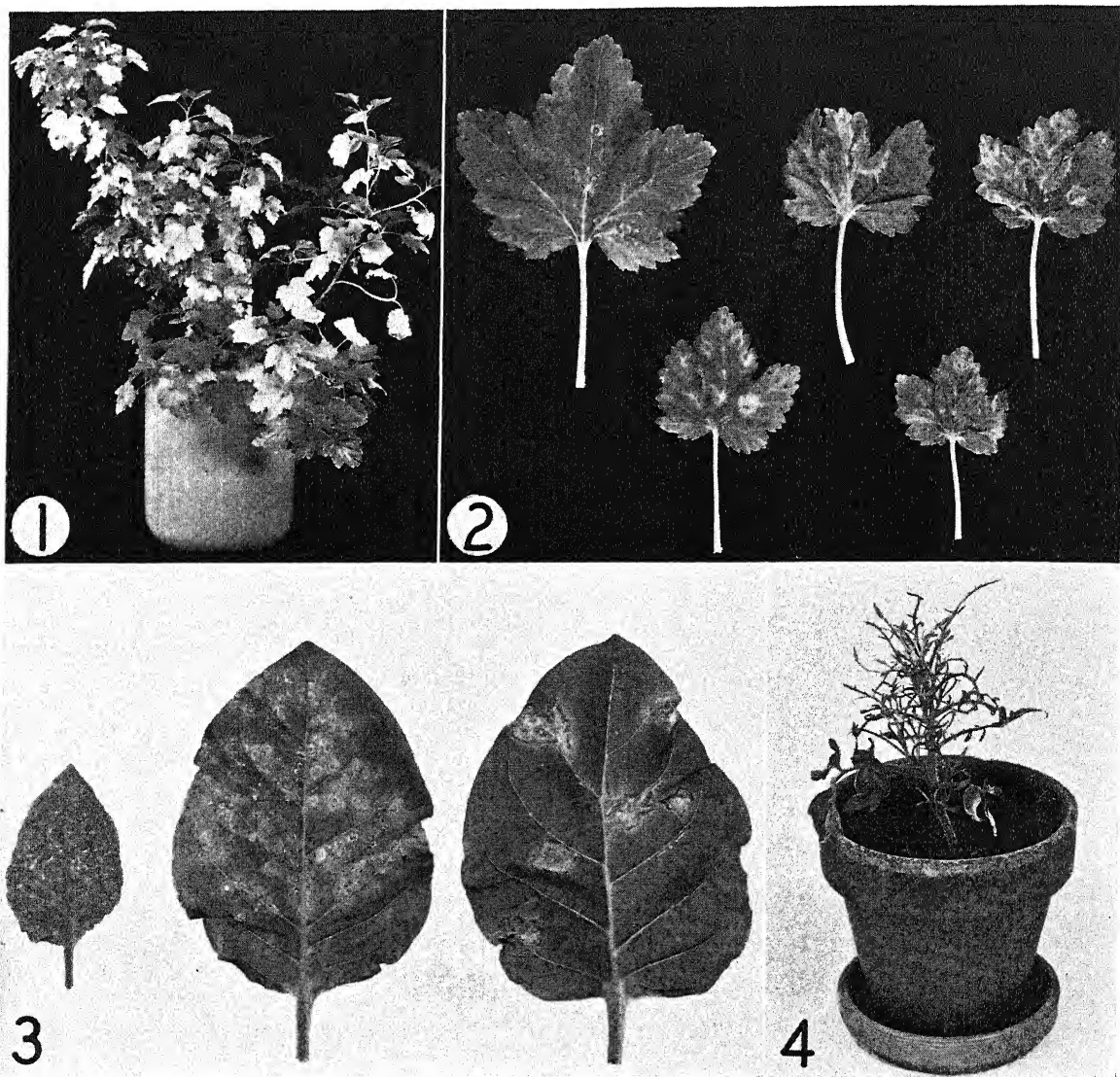


Fig. 1-4.—Fig. 1. Plant affected with an advanced stage of currant mosaic. This plant was dug up from a currant planting in the vicinity of Princeton, New Jersey, and was much stunted in comparison with neighboring healthy plants.—Fig. 2. Symptoms consisting of zonate chlorotic spots occurring on occasional leaves of mosaic diseased currant grown in a greenhouse held between 70° and 80°F. At this temperature currant mosaic symptoms failed to appear. This symptom has not been observed in the field.—Fig. 3. Symptoms consisting of zonate necrotic spots are characteristically produced on Turkish tobacco by the currant strain of tomato ringspot virus. The small leaf on the left shows systemic lesions, while the other two on the right show typical lesions when many or few are produced on tobacco leaves.—Fig. 4. Tomato plant severely stunted by currant ringspot virus as the result of inoculation when about three inches tall. This photograph was made five weeks after inoculation. (Photographs by J. A. Carlile.)

TABLE 1. *Thermal inactivation of currant-ringspot virus in relation to time and temperature of exposure.*

Constant temperature				Constant exposure (10 minutes)	
55°C.		60°C.		Tempera- ture (0°C.)	Ave. lesions ^a per plant (No.)
Exposure (minutes)	Ave. lesions ^a per plant	Exposure (minutes)	Ave. lesions ^a per plant		
0	00 ^b	0	00 ^b	55	156
10	156	1	39	56	50
20	27	2	29	57	13
30	7	3	18	58	7
40	1	4	12	59	2
50	0	5	7	60	<1
..	..	6	4
..	..	7	4
..	..	10	0

^a Four Turkish tobacco plants were employed for each sample of juice.^b Too many lesions to count.

lings, and on rooted cuttings from healthy seedlings, but no symptoms occurred on the plants immunized against tomato ringspot. This test was repeated twice with identical results. Usually four plants falling into each category were employed in each experiment.

Reciprocal tests using plants which had recovered from the currant ringspot virus always gave symptoms when inoculated with tobacco ringspot but showed protection when inoculated with both tomato-ringspot and the currant ringspot viruses. Based on preliminary experiments on virus concentration in juice extract the plants immunized against the currant virus were found to contain approximately as high a virus content as plants with symptoms before recovery; however, this point has not been precisely determined. These tests furnish what is considered conclusive proof that the virus isolated from currant is very closely related to tomato-ringspot virus. Because of its greater virulence it is considered not exactly identical with, but a strain of, the tomato-ringspot virus.

Thermal inactivation.—The currant-ringspot virus became inactivated after an exposure of ten minutes at approximately 60°C. (table 1). The procedure and apparatus for making these determinations were those of Price (1940b). Both Turkish tobacco and the Black variety of cowpea were used for test plants. The two primary leaves of each of eight cowpea plants and of each of four Turkish tobacco plants were inoculated with a single virus sample. Inoculations were made by rubbing a cheesecloth pad soaked in the virus sample on the upper surface of test-plant leaves previously dusted with carborundum. Because the injury caused by carborundum interfered with making readings on the cowpea, these data were omitted from the table.

In a preliminary experiment on thermal inactivation it was found that the fresh virus extract from tobacco failed to survive exposures of one to seven minutes in a water bath held at 70°C. but survived a 55°C. temperature at all intervals up to and includ-

ing thirty-five minutes, the longest period tested. Ten-minute exposures inactivated the virus between 60° and 75°C. but not at 55°C. and below. The second thermal inactivation experiment gave confirmatory and more precise results (table 1). These results agree closely with those of Smith (1937b) for the tomato-ringspot virus, who found that it was inactivated by a ten-minute exposure in water held at 60°C. but not by ten minutes at 55°C.

Dilution end point.—The dilution end point of juice freshly extracted from diseased tobacco was approximately 1:1,000, or slightly above, but never reached as high as 1:5,000. Three dilution end-point experiments were conducted and in every case lesions were obtained on the test plants from the 1:1,000 dilution but never from the 1:5,000 or 1:10,000 dilutions, the number of lesions per test plant averaging three. This concentration of virus averages slightly lower than that usually obtained from diseased currant plants.

Resistance to aging.—The currant-ringspot virus resists between three and four days' exposure in extracted sap at room temperature and a period of longer than one month when held in a mechanical refrigerator maintained at approximately 5°C. For example, one 29-day-old sample held in the refrigerator produced between ten and fifteen lesions per test plant, whereas part of the same sample kept at room temperature had completely lost activity at the end of four days.

The virus in infected leaves resisted freezing and retained activity for at least three weeks when stored in the frozen state.

Passage through a Berkefeld filter removed between three-fourths and nine-tenths of the activity of the virus, based on the local-lesion count on Turkish tobacco.

As a result of the above studies it has been concluded that the strain of ringspot virus isolated from the currant is the same as, or closely related to, that of tomato ringspot.

DISCUSSION.—It is of considerable interest that previous to this study the tomato-ringspot virus had not been found in nature, having appeared on tobacco in the greenhouses at Princeton in the spring-time as a contamination on tobacco presumably brought in by an insect vector. Doubtless there must be wild hosts which are far more important as reservoirs of this virus than the currant. The fact that a virulent strain of tomato ringspot virus was obtained by mechanical means from some and not from other currant plants affected with mosaic definitely indicates that two entities were involved. Failure to transmit the ringspot virus back to the currant by mechanical means may be due to the fact that present methods are inadequate for inoculating currants with viruses or that the insect vector alone is capable of performing such transmissions. This phase of the problem deserves and is receiving additional attention.

SUMMARY

A virus has been recovered in the juice extract from currants which produces a disease on tobacco practically identical to tomato ringspot (Holmes, 1939). This currant-ringspot virus is considered a strain of tomato-ringspot virus, because it agrees with the latter in all important particulars, with the possible exception of being more virulent.

Cross-immunization tests with other ringspot viruses have resulted in protection only against tomato ringspot.

The host range, as far as tested, closely agrees with that of tomato ringspot. Likewise, there is close agreement in other details, including incubation period, thermal inactivation, longevity *in vitro*, filterability, and dilution end-point.

This appears to be the first case of mechanical transmission of a virus from a woody plant to tobacco, but unfortunately thus far it has not been

possible to return the virus to the currant by mechanical means.

Since it has been found that the ringspot virus can be obtained from some and not from other currant plants affected with mosaic, it is tentatively concluded that two entities are involved, currant mosaic and tomato ringspot.

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COMPETITION OF WESTERN WHEAT GRASS WITH RELICT VEGETATION OF PRAIRIE ¹

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MANY PROFOUND changes in mid-continental grasslands have resulted from the recent extended drought. In the prairies of eastern Nebraska, Kansas and South Dakota, the earlier continuous stands of bluestem (*Andropogon scoparius* and *A. furcatus*) have often been greatly fragmented and now occur as relict patches a few square feet to many square rods in extent. Loss of approximately 95 per cent of little bluestem has left the prairie cover very open. Over large areas, except for weeds and a few surviving grasses and forbs, the soil was bared (Weaver, Stoddart and Noll, 1935; Weaver and Albertson,

1939). Grasses more xerophytic than the bluestems have greatly increased, notably needle grass (*Stipa spartea*), prairie dropseed (*Sporobolus heterolepis*) and western wheat grass (*Agropyron smithii*). The wheat grass has entirely replaced other grasses in many prairies and is such a vigorous competitor for water that its invasion into countless relict areas of weakened bluestems has gradually resulted in their disappearance together with most of the accompanying forbs.

It is the purpose of this paper to discuss the ecological characteristics of western wheat grass, to describe the effects of its competition with relict prairie vegetation, and to present experimental data which show that this grass profoundly modifies the water relations of prairie soil.

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GROWTH HABITS AND ECOLOGICAL CHARACTERISTICS.—Western wheat grass (*Agropyron smithii*, Rydb.) is a perennial, sod-forming grass with abundant rhizomes. The rigid, erect stalks vary from 1 to 5 feet in height, depending upon soil fertility and water supply. Stems arise singly or in small numbers from the nodes of the rhizomes, which are usually 1 to 3 inches apart. When the stalks have developed the spikes, which are 2 to 6 inches long, the plants give the mass effect of planted stands of wheat (fig. 1). The rigid, upright, bluish green leaves vary from 4 to about 12 inches in length. They are deeply ridged above and roll tightly inward during periods of drought. Where seasonal moisture is plentiful, the stems grow thickly, often 1,200 or more per square meter, and the sod is compact.

Growth is renewed in true prairie early in spring. The time varies with the season, but it is three to six weeks before the middle of April, in eastern Nebraska, at which time new shoots of little bluestem (*Andropogon scoparius*), big bluestem (*A. furcatus*), side-oats grama (*Bouteloua curtipendula*), and most other grasses begin to appear. A height of 6 to 10 inches is often attained by April 15. Vegetative growth is usually complete and spikes are produced during June, at least on dry years. Maturity is normally attained by mid-July. If the soil becomes dry, western wheat grass is dormant until fall rains stimulate new growth. Otherwise, the leaves remain green and active. Under grazing, growth occurs usually throughout the summer. Since the grass is of northern extraction, it remains green and develops vigorously even late in autumn.

Seed is produced abundantly in true prairie, but the plants also spread widely by means of rhizomes. These are 0.5 foot to more than 3 feet long, much branched, and grow very rapidly. The growth periods of rhizomes are correlated with those of tops. They exhibit great resistance to desiccation (Mueller, 1941).

The roots of *Agropyron smithii* develop rapidly and penetrate deeply. Seedlings near Lincoln, Nebraska, produced roots which extended to a depth of 2.5 feet when three months old (Clements and Weaver, 1924). Many of the roots of mature plants penetrated moist upland prairie soil to a depth of 8 to 9 feet (Weaver, 1920). In the drier soil, the surface absorbing system was much better developed. Numerous, short, horizontal roots arose from the bases of the plants and from the rhizomes. These were profusely branched and rebranched to the fourth order, the ultimate branches being almost microscopic in size and furnishing a good surface absorbing system (Weaver, 1919). Here, most of the roots did not exceed 5 to 6 feet in depth.

Because of the persistence of the rhizomes even under cultivation, western wheat grass is usually the first perennial grass to appear in quantity on abandoned dry-farm lands (Judd and Jackson, 1939; Tolstead, 1941). It is also an early invader, frequently the first, into soils that have drifted over other vegetation causing its death. It may extend up-

ward through a layer of 5 to 10 inches of wind-blown soil.

Increase in abundance of this species was one of the most striking phenomena of the drought (fig. 2). Extensive earlier studies in the prairies of eastern Nebraska, Kansas, and South Dakota (Weaver and Fitzpatrick, 1934) have shown that western wheat

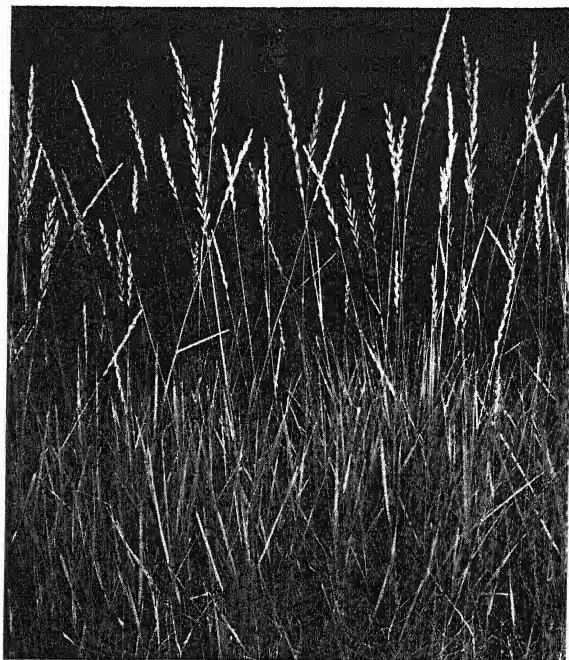


Fig. 1. Characteristic stand of western wheat grass as it appeared in July. The foliage is about 18 inches tall and the flower stalks about 2.5 feet. Photo. by W. L. Tolstead.

grass constituted an almost negligible part of this grassland. Usually it occurred only where there had been some disturbance.

EFFECTS OF COMPETITION ON OTHER GRASSES AND FORBS.—Outcome of competition of wheat grass with other species for water is shown not only in their wilting and dwarfing but also often by their death. Retardation in growth was so common and so striking that numerous measurements were made of the height of the same species growing within areas dominated by western wheat grass and a few feet distant in areas of relict prairie grasses. Average heights in June, 1938, were as follows: tall dropseed (*Sporobolus asper*) 10 and 14 inches, big bluestem 7 and 11 inches, side-oats grama 6 and 7 inches, prairie dropseed 8 and 12.5 inches, and needle grass 13 and 17 inches. Similar differences were found in many prairies; often the suppressed plants were wilted and drying. Usually needle grass was not found with western wheat grass, but where it did occur its flower stalks were frequently reduced to 1 to 6 per plant. June grass (*Coeleria cristata*) was affected in a similar manner (fig. 3 and 4). The mesic Kentucky blue grass, which still grew as a drought



Fig. 2. Northeast hillside of former bluestem prairie near Lincoln, Nebraska, now completely dominated by western wheat grass. July 19, 1940.

relict in bluestem prairie, was never found under wheat grass.

That competition for water was also severe between the individual plants of wheat grass was shown by their growth in dry years. They headed sparingly where this grass had occupied the soil for

several summers, but in adjacent, newly invaded territory where the stems were fewer and competition for water less severe they formed spikes and produced abundant seed. Where western wheat grass was best developed in typical prairies in 1940, the foliage was 22 inches tall and flower stalks 36 inches

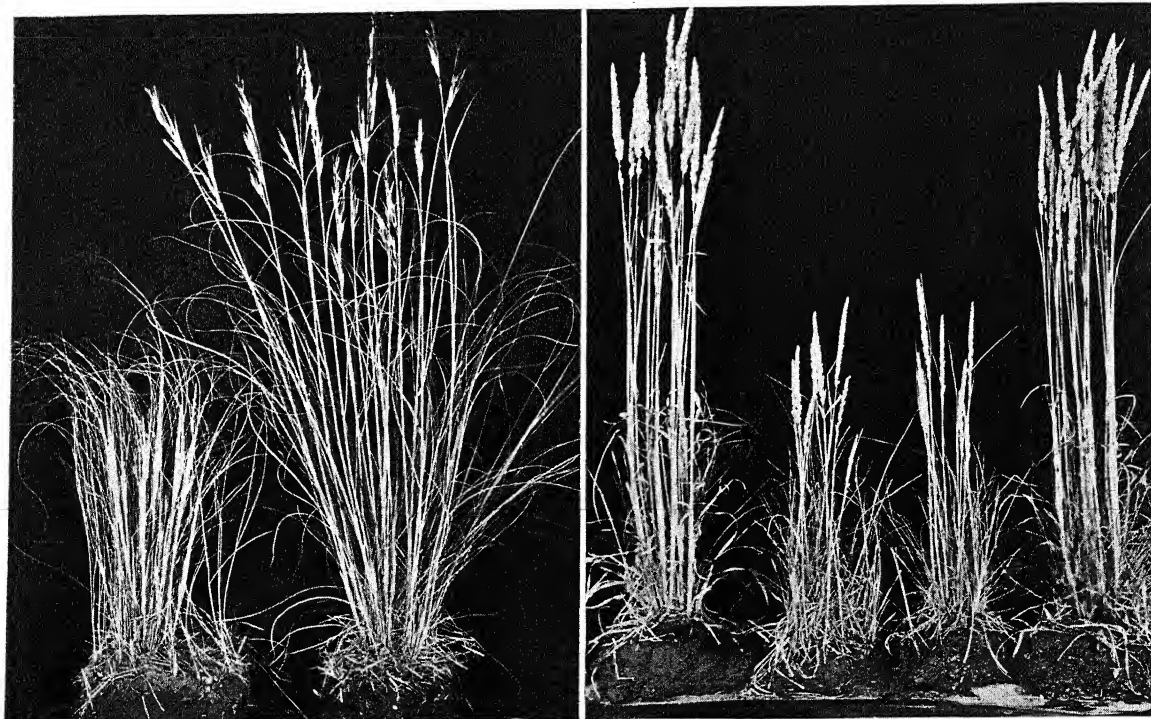


Fig. 3-4.—Fig. 3 (left). Needle grass 13 inches tall growing with western wheat grass near Crete, Nebraska, and a bunch 30 inches high growing a few feet distant without similar competition. The first had dried without producing seed. June 15, 1940.—Fig. 4 (right). Much dwarfed June grass growing with western wheat grass (center) and bunches a few feet distant from prairie uninvaded by wheat grass. June 15, 1940.

high. This contrasted sharply with other, older alternates where the height of the grass was only 15 inches and the flower stalks, if any, were only 22 inches tall.

Even casual observation revealed a striking decrease in number of forbs just as soon as one entered an area dominated by western wheat grass.² Smooth goldenrod (*Solidago glaberrima*) and many-flowered aster (*Aster multiflorus*) have been unusually abundant since the drought, but where they have been invaded by western wheat grass, both have occurred as scattered single-stemmed individuals, or have formed very open patches much reduced in height. Many deeply rooted forbs, such as *Psoralea floribunda*, have been found to be smaller in size in areas of wheat grass, and to have a greatly reduced number of blossoms.

Decrease in both species and numbers of forbs was ascertained in several prairies by counting the perennial forbs in circular areas 50 feet in diameter. These circles were located at random, usually two in patches of wheat grass and two in the prairie grasses where invasion had not occurred. Since it was impossible to determine individual plants of many species with branched rhizomes, and since suppressed plants usually had fewer stems than vigorous ones, the number of stems rather than the number of plants was counted. This could be done accurately by separating the circle into small sectors and examining each individually.

The results of this survey showed that in every sample both the number of species and the number of stems was much lower in areas occupied by wheat grass. Number of species of perennial forbs in western wheat grass varied from 7 to 11 per circle (1,963 sq. ft.) with an average of 9. In areas occupied by other grasses the number ranged from 11 to 24 with an average of 16. The average number of stems in the same sequence was 748 and 3,831, respectively. The largest number of stems in any circle in western wheat grass was 1,566. This was somewhat more than half of the smallest number of stems (2,733) found in any circle in the bluestem grasses. This extensive survey of forbs revealed that competition of wheat grass had reduced the number of species to 56 per cent of those remaining in bluestem and the number of stems to approximately 20 per cent.

Single stems of old plants were frequently found in the wheat grass. These included *Baptisia leucophaea*, *Echinacea pallida*, *Kuhnia glutinosa*, *Petalostemon candidus*, *Psoralea argophylla* and *P. floribunda*. Species most commonly found and with the largest number of stems were *Amorpha canescens*, *Liatis punctata* and *Aster multiflorus*. But even these deeply rooted xeric forbs were often greatly dwarfed, wilting, or dying in the wheat-grass sod.

DRYING OF THE SOIL.—Western wheat grass, by its early and vigorous development, may absorb from

² Loss of forbs has been very high in all grassland west of the Missouri River (Weaver and Albertson, 1939, 1940). The losses have increased with each succeeding year of drought. They will be discussed in a separate paper.

the soil all available water. During years of low precipitation this may occur before later growing species produce much new growth. They can then develop only slowly if at all, depending upon the current rainfall. Moreover, even in good, uniform stands of western wheat grass, the soil surface may be almost without debris. In much of the wheat-grass prairie, the bare soil is exposed directly to sunshine, the shade pattern covering less than one-third of the surface at 10 A.M. At best, only about one-third of the soil is protected by a thin layer of debris and water loss by evaporation directly from the soil is high. The losses by transpiration and evaporation rapidly reduce available water content. Moreover, direct loss of rainfall by runoff is high, since rate of water infiltration is low. These conditions have been repeatedly shown by soil sampling, by field tests on rate of water infiltration, and by experiments on time and amount of water absorption by western wheat grass and other grasses.

Field tests of soil moisture.—Western wheat grass invaded the prairie near Lincoln that has been used several years for experimental studies (Fredricksen, 1938; Noll, 1939). By 1937 it had become well established in numerous large patches. This grass had so depleted the soil water during the dry spring and early summer of 1938 that by July 21 the leaves were rolling and some were beginning to dry. Outside these places the bluestems and other grasses, which resumed growth much later, were unwilted and growing vigorously. Soil samples were taken in duplicate in places occupied by western wheat grass and bluestems, respectively. The pairs of samples within and without the western wheat-grass alternates were taken only 9 feet apart. The soil in any pair of samples was so similar that no difference except that of water content could be found. The excess water content of the soil covered with bluestem prairie over that clothed with western wheat grass is shown in table 1. There was always a higher water content, often 3 to 8 per cent higher, in the bluestem prairie.

TABLE 1. Percentage of water, based on dry weight of soil, in prairie with bluestem vegetation in excess of that in the western wheat-grass type.

Depth, ft.	July 21, 1938			May 2, 1941		
	Sta. 1	Sta. 2	Sta. 3	Sta. 4	Sta. 5	Sta. 6
0 -0.5.....	4.7	3.1	1.3	11.4	2.3	5.2
0.5-1.....	3.6	5.1	4.4	10.5	2.4	2.1
1 -2.....	3.7	7.9	9.2	13.0	2.4	5.1
2 -3.....	4.8	8.1	4.6	10.7	6.4	10.0
3 -4.....	5.8	8.7	2.8	10.4	5.9	6.9

Samples were also taken on May 2, 1941, just within and outside of three different alternates of western wheat grass in the same prairie. Since 1938, however, all of the wheat-grass alternates had greatly extended their area. The depths of moist soil in inches in bluestem prairie and in western wheat grass in the three sets of samples were 48 and 22; 32 and

23; and 47 and 23 inches, respectively. At greater depths the soil moisture was nonavailable, and the grass roots were dead. The samples again showed consistently smaller amounts of water in the wheat-grass soils, often 5 to 10 per cent less (table 1). These examples are representative of many others; at every sampling throughout several seasons the soil under wheat grass was always drier than under the less xeric prairie grasses.

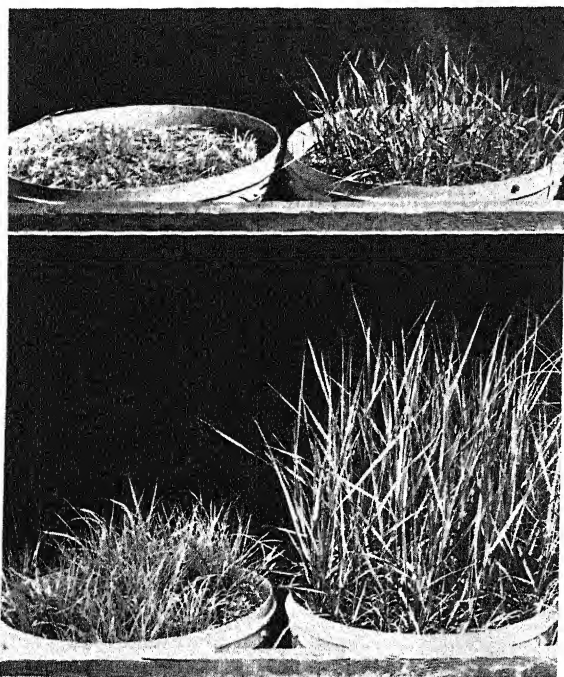


Fig. 5. (Upper.) Development of grasses on April 25. New shoots of *Andropogon scoparius* (left) average an inch in height but only half of the sod is green. Western wheat grass (right) is 7 inches tall and in the fourth-leaf stage. (Lower.) Development on May 20. Little bluestem is only 6.5 inches tall; western wheat grass is 20 inches high and in the eighth-leaf stage; the spikes are just appearing.

Experiments on water infiltration.—The rate at which water enters prairie soil has been shown experimentally to be determined largely by the cover of grass and debris. Cover prevents loosening of the soil by the impact of the water and carrying of the finer soil particles into the soil pores, thus more or less closing them near the soil surface. On soils bared by drought and then invaded by western wheat grass, intake of water may be reduced by the formation of a thin, dense, compact layer on the surface (Duley and Kelly, 1941). The rate of entry of water was determined for soil covered with bluestem or other predrought native grasses and for the same soil clothed with western wheat grass. In these experiments, steel cylinders, one square foot in cross-sectional area and 4 inches long, were used. The cylindrical wall was only 2 mm. thick, and the steel was sharpened; hence, when it was oiled it

could easily be forced vertically into the soil without disturbance to soil structure to a depth of 3.75 inches. Water was then added, as rapidly as it could be absorbed, from a sprinkling can with small perforations until a total of one gallon had been applied. The time for the infiltration of the water was recorded. Data were obtained from eight widely separated prairies; the first six were in southeastern Nebraska and the last two in north-central Kansas. All were on soils of silt-loam texture. The pairs of experimental areas were never more than 9 feet apart and sometimes only 6 feet. Prairie grasses were predominantly big bluestem (table 2).

TABLE 2. Minutes required for the infiltration of one gallon of water sprinkled slowly on one square foot of soil covered with (P) predrought prairie grasses or (W) western wheat grass.

Station	P	W
Lincoln	6.5	30.0
Lincoln	6.5	27.5
Pleasant Dale	7.5	13.5
Pleasant Dale	4.0	6.5
Carleton	8.0	21.0
Carleton	11.5	16.1
Hebron	6.0	9.0
Nelson	5.0	18.0
Clay Center	3.0	7.5
Clay Center	5.5	10.5
Montrose	5.0	12.5
Belleville	8.5	11.5

The time of infiltration of water was consistently much greater in western wheat grass. The average time was 6.4 minutes where a stand of prairie grasses was present and 15.3 minutes where western wheat grass clothed the soil. Thus, the rate of infiltration was 2.4 times as rapid in normal prairie as in western wheat grass. This accounts in part for more moist soil in undisturbed prairie and drier soil in wheat grass. Similar differences in depths of water penetration after rains have been repeatedly observed.

In another experiment at Lincoln, Nebraska, water was added to two separate plots of predrought prairie grasses, each 3×5 feet in area, and two adjacent ones of western wheat grass on nearly level land. Six-inch boards were placed on edge, 5 inches deep, firmly around each plot to prevent runoff. Water was added to the dry soil from sprinklers for 1.5 hours as rapidly as it was absorbed. Average absorption in the bluestem plots was equivalent to 7.27 inches of rainfall, that in the wheat-grass plots amounted to only 2.98 inches. Soil in the undisturbed prairie was wet to an average depth of 29 inches at the end of the experiment; that under wheat grass to only 10.4 inches. This again illustrates the degree to which infiltration of water into soil covered with wheat grass is retarded.

Experiments on water loss.—Early in the spring of 1941, large blocks of sod of western wheat grass and little bluestem were secured in single pieces to a depth of 10 inches. They were circular in area and

just large enough to fit into galvanized iron containers 18 inches in diameter. These containers, which were 24 inches deep, had previously been filled to within 12 inches of the top with well tamped, fertile, silt-loam soil of optimum water content. Two sods of each species were transplanted, care being taken to maintain the natural surface mulch. Soil was packed firmly about them so that after moistening no water was lost through spaces which might have occurred had the soil shrunk from the wall of the container after transplanting. The soil level was 2 inches below that of the top of the container, thus permitting absorption of rainfall without runoff. The containers were placed out-of-doors in a frame of two-inch planks, the interspaces between containers and frames being filled with insulating material. The initial water content was approximately maintained by restoring the containers to their original weight by the addition of water at each of the 6 weighings (or oftener if necessary) between March 25 and May 20. Water received directly from rainfall was measured by means of a rain gauge placed beside the containers.

Spring was very late and cool. Western wheat grass began growth on April 1 but little bluestem remained dormant until April 15. Some water, of course, was lost by direct evaporation even from the containers of dormant little bluestem (fig. 5).

The greater loss of water (*i.e.*, transpiration plus evaporation) from the early maturing western wheat grass compared with that lost from little bluestem was very marked. That from wheat grass was 86.65 pounds, which was more than twice as much as that from little bluestem, 41.55 pounds. Experiments in prairie at Lincoln, Nebraska, during the preceding spring are in accord with these findings (R. J. Weaver, 1941).

Rapid development in early spring and early maturity is an excellent method of evading summer drought. Consequently, western wheat grass competes very successfully with prairie grasses of later growth, especially during years of low rainfall.

ROOT DEPTH DURING DROUGHT.—The depth of the root system of western wheat grass was examined at numerous stations near Lincoln in 1941, as was also the depth of big bluestem growing near it. Usually a single trench 7 to 8 feet long permitted the examination of both species. A layer of subsoil 1.5 to 3 feet thick and beginning at a depth of 2 to 3 feet had been without available water over a period of several years. The spring of 1941 was one of rather high rainfall, however, and the surface soil to a depth of 2 to 3 feet had been well moistened. On May 30 and 31, the roots of wheat grass reached a depth of only 24 inches, below which the silt-loam soil was without water available to growth. Roots of big bluestem, 3 feet distant, extended to the depth of moist soil which was at 33 inches. In another excavation, also in silt-loam soil, maximum root depths were at 24 and 48 inches, respectively. In a trench excavated in a third prairie, the maximum depth of the extremely dense network of wheat-grass roots was 30 inches,

but that of big bluestem and certain forbs was 50 inches. That little and big bluestem and prairie forbs had been the former occupants was clearly revealed by the abundance of their dead and partially decayed roots which were found intermixed with those of the living wheat grass but which also occurred in the dried soil two or more feet below the wheat grass.

Despite the shallowness of soil moisture, western wheat grass was in excellent condition. The foliage was 20 inches high and the flower stalks 10 to 12 inches taller.

PRACTICAL SIGNIFICANCE.—Western wheat grass has a much lower forage value in true prairie and in native pastures originating from true prairie than the better forage grasses which it is able to suppress or which often die as a result of competition for the limited water supply during drought. Hence, its tendency to form pure stands or a nearly pure growth at the expense of most other species creates a serious economic problem. Only two native grasses compete successfully with it. Side-oats grama has done so only when early spring was dry and late spring and summer moist. Blue grama is more drought resistant than western wheat grass. It successfully invades pure stands of this grass and may gradually replace it. But blue grama is a short grass and yield is relatively low.

SUMMARY

Agropyron smithii is a common sod-forming, perennial, forage grass of midwestern prairies. It is so successful a competitor for the meager supply of soil moisture that it often causes the death of more mesic grasses and forbs of the true prairie. It renews growth in early spring, produces abundant foliage which normally reaches a height of 1.5 to 2 feet in June and is overtopped by flower stalks 1.5 to 2 feet taller. Seed is produced in abundance, and migration is rapid by means of long, slender, much branched rhizomes. Formerly occurring sparingly in the eastern portions of Nebraska and Kansas, western wheat grass spread rapidly and widely following the great deterioration of grassland due to drought.

The early luxuriant growth, when water was available, resulted in greatly reducing the amount of soil moisture for use by other species, most of which began development four or more weeks later. Lack of much debris under western wheat grass permitted rain to loosen the surface soil and roil the water that entered it. This resulted in decreased infiltration and greater runoff than on soil covered with bluestems. Amount of water transpired from a normal stand of western wheat grass or evaporated from the soil it covered was more than twice as great from March 25 to May 20 as that from little bluestem prairie.

Normal root depth in moist soil is about 8 feet; during the drought the depth corresponded with depth of moist soil which was about 2 to 2.5 feet. A few feet distant, higher water content and much deeper penetration of both water and roots of other grasses were recorded.

Competition for water resulted in great dwarfing and often in wilting and death of most other prairie grasses and forbs. Numbers of species and numbers of stems of perennial forbs were greatly decreased after western wheat grass once became thoroughly established. In prairies on silt-loam soil and under similar precipitation, the number of perennial species of forbs was only 56 per cent as great in wheat grass, and the number of stems 20 per cent of that in uninvaded areas.

The large area of drought-damaged true prairie and native pasture now dominated by western wheat grass and the harmful effects of the successful competition for water of western wheat grass with species of greater forage value present a problem of much scientific interest and great economic importance.

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THE LIGHT-CONTROLLED DIURNAL RHYTHM OF ASEXUAL REPRODUCTION IN *PILOBOLUS*¹

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FOR OVER two and one-half centuries botanists have been interested in *Pilobolus*. They have investigated this delicate coprophilous representative of the Mucorales in relation to its taxonomy, morphology, phototropism, and periodicity. However, only the first three aspects have received detailed treatment. The most striking property of *Pilobolus*—rhythmic asexual reproduction—has not been accorded careful study.

It will be evident from the survey of literature to follow that little more is known concerning this periodicity than that it is effected by, or at least influenced by, the alternation of day and night. Most of the knowledge thus far acquired has been incidental to study of other aspects of the behavior of *Pilobolus*, particularly the influence of light on sporangiophore development. Past investigators have not examined

the periodicity directly, possibly because the technique of growing *Pilobolus* in cleared nutrient agar was not perfected. Since this culture method greatly extends the possible range of experimentation, a reopening of the problem of periodicity of asexual reproduction in *Pilobolus* seems justified. This paper (1) presents evidence that periodicity is wholly dependent upon daily alternating periods of light and darkness, and (2) contains observations indicating an experimental approach to the problem of the mechanism controlling periodicity.

HISTORICAL BACKGROUND.—The earliest known reference to *Pilobolus* appeared in John Ray's "Historia Plantarum" in 1688. However, mycological investigations of that period were primarily descriptive in nature, and many years elapsed before the diurnal reproduction of *Pilobolus* was fully described. Conceivably some of the early workers were cognizant of the periodicity, but it is impossible to determine the extent of their knowledge from the three or four line descriptions they gave.

Indeed it was not until 1851 that the phenomenon of periodic asexual reproduction was described in detail. In that year Cohn (1851) set forth the correct chronological sequence of all stages in the life cycle

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of *P. oedipus* Mont. Cohn, however, restricted his investigations to morphology and chronology and, except to express his belief that each fruiting body passes through its entire development within twenty-four hours, made no reference to the effect of external factors on sporangiophore development.

Four years later Bail (1855), comparing the sporangiophores of *P. crystallinus* (Wiggers) Tode and *P. roridus* (Bolt.) Pers., stated that the sporangiophores varied in dimensions according to "different light and air conditions." Although Bail presented no experimental data, his observations may be regarded as the first to relate reproductive periodicity in *Pilobolus* to the environment.

It is Eugène Coemans (1861) to whom we are indebted for the discovery that light is the primary factor in regulation of the life cycle of *Pilobolus*. In his "Monographie du Genre *Pilobolus*" Coemans offered the following original observations: (1) cultures kept in continuous darkness lose their periodicity; (2) in continuous darkness the sporangiophores, delaying sporangium formation, become greatly attenuated; and (3) the time of sporangium projection is directly dependent upon light. It is a well earned tribute to Coemans' powers of observation and analysis that since his researches only one major contribution (that of Gräntz) has been made to the problem of periodicity of reproduction in *Pilobolus*. The investigations of Klein (1872), Brefeld (1881), Grove (1884, 1934), and Buller (1934) contributed little to advance understanding of the factors involved in rhythmic reproduction. Brefeld (1881) made a fairly detailed study of sporangiophore behavior in continuous darkness, but this work was no more than an extension of Coemans'.

The last paper of significance is that of Gräntz (1898). Gräntz's most important observation was that not only continuous darkness, but continuous light also, causes a cessation of reproductive periodicity. With this work, research on factors influencing periodicity subsided. Subsequent authors were content merely to state that asexual reproduction in *Pilobolus* is periodic through the agency of light.

MATERIALS AND METHODS.—Of the many papers dealing with the culture of *Pilobolus* less than half-a-dozen contain reports of successful culture of this fungus in nutrient agar. Gräntz (1898), Bersa (1929), Krafczyk (1931, 1935), Swartz (1934), and Buller (1934) obtained adequate growth of mycelium and formation of mature fruiting bodies on dung agar. For the present experimental work the writer has used a 1 per cent dung decoction agar, prepared from a commercial fertilizer ("Bovung," a product of the Walker-Gordon Laboratory, Plainsboro, New Jersey). This nutrient medium, buffered to pH 6.5 by the addition of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ and KH_2PO_4 , each to $\frac{1}{200}$ molar concentration, has been successfully employed for over five years in culturing eight species, and has given consistently excellent growth.

In the experiments designed to test the effect on *Pilobolus* of alternating light and darkness, cultures

were kept in a large Thelco Electric Incubator maintained at 20°C ., in order to obviate the factor of a diurnally fluctuating temperature. Four sixty-watt bulbs provided illumination, which was made of uniform intensity by passing it through a ground glass plate. An electric fan installed in the incubator provided an air circulation sufficiently rapid to prevent a change in temperature when the light went on or off. For reasons to be explained later, cultures subjected either to continuous light or to continuous darkness were kept at room temperature.

Since light exerts a formative or morphogenic influence on the developing fruiting bodies of *Pilobolus*, observation of cultures kept in continuous darkness had to be carried out in light passed through a monochromatic filter (Jena filter #RG 5) which cut out all rays shorter than 6,700 Å. Such light, barely adequate for observation, had no appreciable influence on the developing sporangiophores.

THE LIFE CYCLE OF PILOBOLUS.—For full understanding of the experiments to follow, knowledge of the behavior of *Pilobolus* in both agar and dung culture is necessary. In the latter type of culture the sporangiophores originate during the afternoon from hyphal swellings, or fruiting body primordia, which are either upon or just below the surface of the dung. In the early hours of the evening each sporangiophore enlarges at its apex to form a sporangium. Shortly after midnight the subsporangial swelling begins to appear. In the course of the remaining hours of darkness the fruiting body matures completely, as the spores are cut out and the sporangial wall becomes thickened and blackened. In late morning the sporangium dehiscens, and is soon projected with explosive violence from the sporangiophore, which simultaneously collapses. By mid-afternoon, as a new crop of sporangiophores begins to emerge from recently formed fruiting body primordia, all fruiting bodies have discharged their sporangia. It is important to remember that in dung cultures of *Pilobolus* all sporangiophores are in approximately the same stage of development at any hour of the day or night.

Interpretation of the life cycle of *Pilobolus* in agar culture is greatly facilitated by the fact that the mycelium is completely submerged within the (transparent) medium. This striking habit of growth makes it possible to study hyphal structure in great detail, and to follow the formation and development of fruiting body primordia under high dry magnification. The following description of a life cycle in agar is for *P. Kleimii* van Tieghem, but may be considered representative of the genus.

The fruiting body primordium, or trophocyst, so termed by Morini (1900), arises as the localized accumulation of coarsely granular, orange-red protoplasm. Receiving a continuous supply of protoplasm, the young trophocyst slowly swells (fig. 1a-c) and ultimately is isolated from the mycelium by the formation of two septa (fig. 1d). Under the influence of the still streaming protoplasm, which is now prevented by the septa from entering the trophocyst,

that portion of the hypha immediately contiguous to the proximal septum swells slightly, forming an apophysis (fig. 1e). During apophysis formation the trophocyst absorbs water, presumably through a change in permeability of the walls, and increases in diameter. Simultaneously the trophocyst protoplasm becomes increasingly vacuolate. A mature trophocyst (fig. 1f) is ovate-cylindrical in shape, with a thick peripheral layer of protoplasm and a single large central vacuole.

one-half hours later the subsporangial swelling (fig. 1j) is clearly indicated. The fruiting body is completely formed (fig. 1k) eleven to twelve hours after sporangiophore emergence from the agar. Subsequently the sporangium dehisces, and the fruiting body collapses.

It is evident that the periodicity of asexual reproduction exhibited by *Pilobolus* in nutrient agar is by no means so precise as that exhibited in dung. In the latter type of culture, at any hour of the day

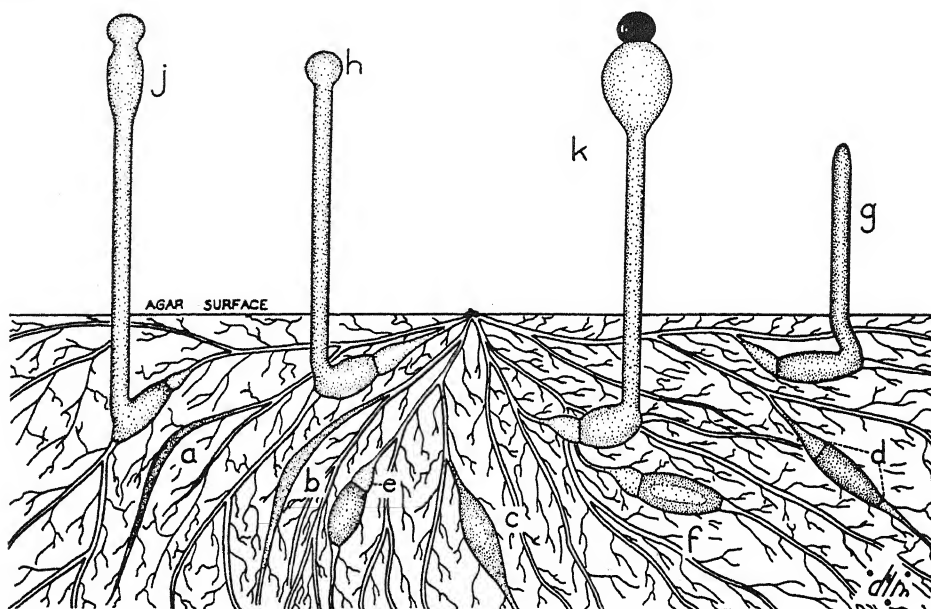


Fig. 1. Schematic diagram of a section through an agar culture of *P. Kleinii*, showing development of trophocysts and sporangiophores; a-c, successive stages in formation of young trophocyst; d, septa isolating trophocyst from subtending hypha; e, apophysis; f, fully mature trophocyst with central vacuole; g, sporangiophore after emergence from agar; h, apical enlargement of sporangiophore to form sporangium; j, beginning of subsporangial swelling; k, mature fruiting body.

The findings of the writer do not corroborate the belief of past workers that trophocyst formation takes place only during the morning, within the comparatively short space of a few hours. On the contrary, trophocysts can be found in all stages of development at any time, and can require as much as forty-eight hours for development. Further development, however, does follow a fairly rigid time schedule. Between 3 P.M. and 5 P.M. a sporangiophore grows forth from the distal end of the trophocyst.

The sporangiophore, which may be considered an extension of the trophocyst since the protoplasm and central vacuole of both are continuous, exhibits marked negative geotropism and positive phototropism. Only after having grown out of the agar (fig. 1g) does the sporangiophore pass through the successive stages of development outlined for sporangiophore behavior in dung culture. The period during which sporangiophores emerge from the agar extends from approximately 3 P.M. to 10 P.M. Formation of the sporangium (fig. 1h) takes place about four to five hours after this emergence. Three and

or night all sporangiophores are in approximately the same stage of development. In agar culture, at any particular moment the sporangiophores can be as many as seven hours apart in their development. The explanation of this striking difference in behavior is to be found in the place of formation of the trophocyst in the two types of culture. In dung culture all trophocysts are formed upon or just below the surface of the dung. The sporangiophores which arise from the trophocysts protrude into the air almost simultaneously, and undergo subsequent developmental changes synchronously. In agar culture, however, trophocysts are formed at different levels within the substratum, and, although the sporangiophores grow forth from these trophocysts simultaneously, formation of sporangia, etc., cannot take place until the sporangiophores have emerged from the agar. Since sporangiophores may emerge from the agar during a period extending from 3 P.M. to 10 P.M., these sporangiophores, following their emergence, may be seven hours apart in development. The time of emergence from the agar of the sporangio-

phore is in part a function of the depth of the trophocyst within the agar. One may, therefore, conclude that in dung culture periodic maturation of fruiting bodies depends upon periodic emergence of the sporangiophore from the trophocyst, and in agar culture periodic maturation of fruiting bodies depends upon periodic emergence of the sporangiophore from the agar.

The writer has found it possible to grow agar cultures of *Pilobolus* in an atmosphere of pure nitrogen, in Kolle flasks. In these completely anaerobic agar cultures there is a striking parallel to the habit of growth in ordinary dung culture: the majority of trophocysts are formed on the surface of the substratum. A consideration of the place of trophocyst formation in the three types of culture (dung, aerobic agar, anaerobic agar) leads logically to the suggestion that *Pilobolus* is a microaerophilic organism. The soundness of this hypothesis is being tested experimentally.

EXPERIMENTS.—Determination of the presence or absence of periodicity in cultures subjected to a particular set of light conditions was most effectively made by a count of the number of sporangiophores in each of five arbitrarily chosen stages of development. Any significant deviation from the standard count, obtained from cultures subjected to normal twenty-four-hour alternation of light and darkness, could then be interpreted as altered sporangiophore behavior. The five stages of sporangiophore development chosen are shown in figure 2. Results of the following experiments are presented in tabular form, showing the number of sporangiophores in each stage at 8 A.M. and at 8 P.M. The number given represents the total for an indicated number of Petri plate cultures. Interpretation of the results has been further simplified by grouping together the percentages and totals of stages I and II (the period of emergence and elongation) as group A, stages IV and V (the period of maturation) as group C, keeping separate stage III (the period of sporangium formation) as group B. This procedure of grouping is justified by the fact that, in cultures which have been subjected to alternating light and darkness, each of the three periods represents an approximately equal length of time (four hours) in the development of the sporangiophore above the agar. Since the experiments were performed with different numbers of Petri plate cultures, and since these cultures varied in age, comparisons of the results of the experiments cannot be made on the basis of the absolute numbers of sporangiophores observed. To obviate this difficulty, the group counts have also been expressed as percentages. The following experiments were carried out with *P. microsporus* Klein.

Normal alternation of light and darkness; Experiment 1.—In order to establish the standard count, thirteen Petri plate cultures were subjected to light from 8 A. M. to 8 P. M., and to darkness from 8 P. M. to 8 A. M., over a period of five days. Counts made of the sporangiophores at 8 A. M. and 8 P. M. each day are given in table 1. Total counts and percentages for

the entire five days of groups A, B, and C were as follows: at 8 A. M.—group A, 1,997 (96.9 per cent); group B, 62 (3.0 per cent); group C, 3 (0.1 per cent); at 8 P. M.—group A, 47 (1.0 per cent); group B, 266 (5.9 per cent); group C, 4,189 (93.1 per cent). These figures were used as standards with

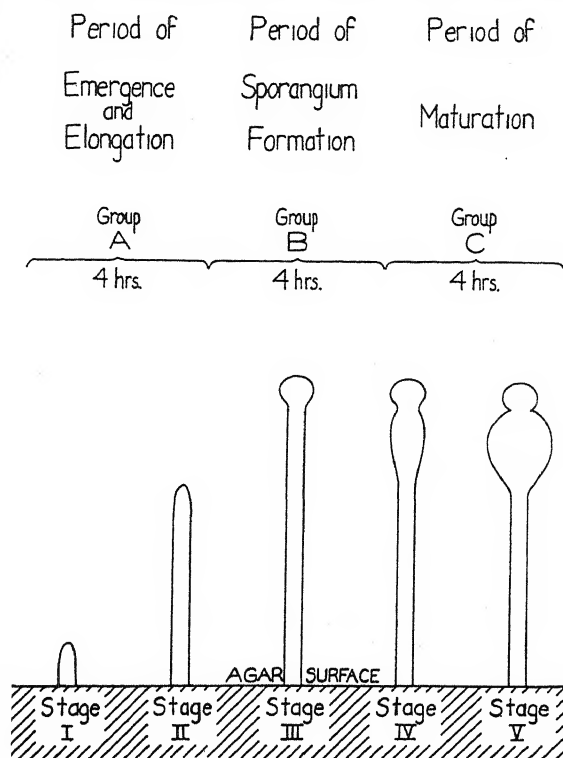


Fig. 2. Diagram showing five stages of *Pilobolus* sporangiophore development above the agar. These stages were arbitrarily chosen for use in determining presence or absence of periodicity of reproduction in *Pilobolus* cultures subjected to various conditions of illumination.

which to compare results obtained from other experiments.

The totals just given for the three groups are of great value in demonstrating the long interval of time during which sporangiophores may emerge from the agar. Thus the presence of 266 sporangiophores in group B at 8 P. M. indicates that sporangiophore emergence from the agar must have begun at least four hours earlier. Furthermore, the number (4,189) of sporangiophores in the period of maturation at the end of twelve hours of darkness is markedly greater than the total number of sporangiophores in all groups (2,062) at 8 P. M., showing that sporangiophores must have continued to emerge from the agar after 8 P. M. The presence of 62 sporangiophores in the period of sporangium formation at 8 A. M. may possibly indicate that sporangiophores which emerge from the agar late in the evening elongate for a longer time before forming sporangia than do sporangiophores which emerged from the agar during the afternoon or early evening. If this were not so,

the last sporangiophores to emerge from the agar (at 10 P.M.) would have formed sporangia by 2 A.M., four hours after emergence, and would be well into the maturation period by 8 A.M.

Reversed alternation of light and darkness; Experiment 2.—If periodicity of asexual reproduction is caused by daily alternation of light and dark periods, a reversal of periods should bring a reversal of periodicity—that is, the majority of sporangiophores should be in group C at 8 P.M., and the ma-

riod, no matter what successive twelve-hour intervals of the day or night the light and dark periods may cover.

Since in both the first and second experiments the cultures of *Pilobolus* were kept at constant temperature and, nevertheless, exhibited periodicity of reproduction, there is strong indication that temperature—the only other external factor which might effect rhythmic formation of fruiting bodies—does not play an important part in periodicity.

TABLES 1-6. *Sporangiophore counts on agar cultures of P. microsporus subjected to various conditions of illumination.*

TABLE 1. NORMAL ALTERNATION

ILLUMINATION	8A.M.	LIGHT					8P.M.	DARK					8A.M.	LIGHT					8P.M.	DARK					8A.M.	LIGHT					8P.M.	DARK				
TIME		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V
STAGE		14	184	3	0	0		3	3	37	57	357		35	318	0	0	0		3	3	20	118	516		28	362	11	0	1		5	9	55	143	577
NUMBER		198	3	0	0	0		6	37	414	353	0	0		6	20	634	410		6	20	634	410	0		28	362	11	0	1		14	55	720		
PER CENT		96.5	1.5	0.0	0.0	0.0		1.3	8.1	90.6	100.0	0.0	0.0		0.9	3.0	96.1	97.2		0.9	3.0	96.1	97.2	2.6		0.2					1.8	7.0	91.2			

TABLE 2. REVERSED ALTERNATION

ILLUMINATION	8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK				
TIME		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V
STAGE		6	40	3	0	0		1	4	3	19	59		19	116	7	0	0		7	0	1	38	156		29	190	13	0	0		6	4	7	73	238
NUMBER		46	3	0	0	0		5	3	78	135	7	0		7	0	1	194		3	0	1	194	219		29	190	13	0	0		10	7	31	31	
PER CENT		93.9	6.1	0.0	0.0	0.0		5.8	3.5	90.7	95.1	4.9	0.0		3.5	0.5	96.0	94.4		1.3	0.0	0.0	94.4	56		0.0					3.1	2.1	94.8			

TABLE 3. CONTINUOUS DARKNESS

ILLUMINATION	8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK						
TIME		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		
STAGE*		36	135	0	0	0		47	325	2	0	0		60	491	0	0	0		55	523	9	14		43	574	42	8		57	621	34	12		52	681	11	14
NUMBER		171	0	0	0	0		372	2	0	0	0		551	0	0	0	0		578	9	14		617	42	8		678	34	12		733	0	0	0	0		

TABLE 4. CONTINUOUS LIGHT

ILLUMINATION	8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK				
TIME		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V
STAGE		20	58	77	16	75		27	70	105	18	86		44	88	110	36	114		59	200	285	45	167		59	182	282	64	290		40	222	361	111	358
NUMBER		78	77	91	0	0		97	105	104	132	110	150		259	258	212	241		259	258	212	241	0		259	258	212	241	0		259	258	212	241	0
PER CENT		31.7	31.3	37.0	0.0	0.0		31.7	34.3	34.0	33.7	28.0	38.3		35.5	35.4	29.1	28.1		35.5	35.4	29.1	28.1	0.0		35.5	35.4	29.1	28.1	0.0		35.5	35.4	29.1	28.1	0.0

TABLE 5. ALTERNATING TO CONTINUOUS LIGHT

ILLUMINATION	8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK				
TIME		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V						
STAGE		1	5	6	80	500		37	370	19	0	0		56	251	490	138	369		23	127	135	51	261		25	108	100	41	74		25	101	118	50	99						
NUMBER		6	8	580	407	19		307	490	507	150	135	312		133	100	115	126		133	100	115	126	118		133	100	115	126	118		133	100	115	126	118						
PER CENT		10	10	98.0	95.5	4.5		23.5	37.6	38.9	25.1	22.6	52.3		38.2	28.7	33.1	32.1		38.2	28.7	33.1	32.1	30.0		38.2	28.7	33.1	32.1	30.0		38.2	28.7	33.1	32.1	30.0						

TABLE 6. CONTINUOUS LIGHT TO ALTERNATING

ILLUMINATION	8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK					8P.M.	LIGHT					8A.M.	DARK				
TIME		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V		I	II	III	IV	V
STAGE		16	42	56	15	22		22	36	40	18	24		0	3	1	16	53		38	161	11	0	0		3	24	17	216	385		66	505	6	0	0
NUMBER		58	56	37	0	0		58	40	42	3	1	69		199	11	0	0		199	11	0	0	0		27	17	216	385	66	505	6	0	0	0	
PER CENT		38.4	37.1	24.5	0.0	0.0		41.4	28.6	30.0	4.1	1.4	94.5		94.8	5.2	0.0	0.0		94.8	5.2	0.0	0.0	0.0		4.2	2.6	93.2	99.0	10.0		99.0	10.0	0.0	0.0	

* Conditions of observation made it impossible to distinguish between stages IV and V.

jority in group A at 8 A.M. The data obtained from such an experiment, and presented in table 2, show that this condition actually obtained. Counts on nine plates for five days gave the following totals and percentages: at 8 A.M.—group A, 1,178 (96.3 per cent); group B, 45 (3.7 per cent); group C, 0 (0 per cent); at 8 P.M.—group A, 30 (2.4 per cent); group B, 19 (1.5 per cent); group C, 1,204 (96.1 per cent).

This single experiment gave definite indication that the periodicity exhibited by *Pilobolus* is not inherent, for it followed that sporangiophores can be caused to mature at any time of the day or night, simply by suitably adjusting the alternating periods of (artificial) light and darkness. If an agar culture of *Pilobolus* is exposed to alternating twelve-hour periods of light and darkness, a majority of fruiting bodies invariably will be found mature at the end of the dark period, immature at the end of the light pe-

Continuous darkness; Experiment 3.—The contention of Coemans (1861), Brefeld (1881), and others, that cultures of *Pilobolus* do not show periodicity when kept in continuous darkness, was corroborated by the results of the third experiment, in which counts were made for four days on thirteen plates subjected to continuous darkness. The results are shown in table 3. These results have not been totaled, since this treatment would detract significance from several important points. These points are: (1) the number of sporangiophores in group A, the period of emergence and elongation, shows a steady increase over the four days; (2) sporangiophores in group B, the period of sporangium formation, do not begin to appear in significant numbers until at least forty-eight hours have elapsed following emergence from the agar; and (3) a minimum of three or more days is required for the entire process

of sporangiophore development. Of particular importance is the fact that approximately equal numbers of sporangiophores are to be found in stage I at each observation time.

Continuous light; Experiment 4.—Since continuous darkness had been shown to prevent periodicity, continuous illumination was then employed. Counts made on twenty-four cultures grown for five days in continuous light (see table 4) yielded the following totals and percentages: at 8 A.M.—group A, 1,171 (26.9 per cent); group B, 1,424 (32.7 per cent); group C, 1,758 (40.4 per cent); at 8 P.M.—group A, 989 (27.7 per cent); group B, 1,104 (30.9 per cent); group C, 1,477 (41.4 per cent). These results show that at any time of the day or night significant numbers of sporangiophores can be found in all three periods of development. The only point of agreement between the results of the experiments with continuous light and continuous darkness is that, in both, counts for stage I indicate that sporangiophores emerge from the agar irrespective of the time of day or night. In continuous darkness the sporangiophore continues to elongate for many hours before forming a sporangium; in continuous light sporangium formation takes place within a very short time after the sporangiophore has emerged from the agar—a fact which can be determined also from measurement of the average length of mature fruiting bodies.

The last two experiments (conducted at room temperature) gave further evidence of the unimportance of temperature for the periodic formation of mature fruiting bodies. Room temperature was allowed to fluctuate between 20°C. during the day and 13°C. during the night. This daily fluctuation had no observable effect on the cultures. At each observation time sporangiophore counts indicated continuing lack of periodicity.

Alternating light and darkness followed by continuous light; Experiment 5.—With alternating periods of light and darkness firmly established as the cause of periodicity of reproduction, the next step was to determine how long a period of continuous illumination was necessary to cause cultures which had been subjected to alternating light and darkness to lose their periodicity. The nine *Pilobolus* cultures of the second experiment were used for this purpose. At the end of the last period of illumination, when the cultures showed the usual high percentage of sporangiophores in group A (the period of emergence and elongation), the light was allowed to remain on for forty-eight more hours. The results of observations made at succeeding twelve-hour intervals during the extra two days of illumination appear in table 5, together with the counts which had been made during the preceding twenty-four hours. Periodicity disappeared within twelve hours, as shown by the percentages obtained at 8 P.M., at the end of the first twelve hours of extra illumination: group A, 23.5 per cent; group B, 37.6 per cent; group C, 38.9 per cent. This distribution is characteristic of cultures growing in continuous light.

The extremely short interval of time sufficient to cause loss of periodicity under these conditions depends upon the fact that the sporangiophores continue to emerge from the agar when the illumination is extended, rather than ceasing to emerge at the end of the usual seven-hour interval.

Continuous light followed by alternating light and darkness; Experiment 6.—The results obtained by reversing the conditions of the preceding experiment are given in table 6. Here eight plates which had been growing in continuous light were subjected at 8 A.M. to twelve hours of darkness, followed by twelve hours of light, and so on. Again, twelve hours were sufficient to establish a sporangiophore count characteristic of cultures exposed to alternating light and darkness. Thus at 8 P.M. the percentages were as follows: group A, 4.1 per cent; group B, 1.4 per cent; group C, 94.5 per cent. This percentage distribution obtained because the sporangiophores had ceased to emerge from the agar at 10 A.M. (the usual two hours after illumination had stopped).

Continuous darkness followed by alternating light and darkness; Experiment 7.—Somewhat the same phenomenon was observed when continuous darkness was used in place of continuous light. Although experiments were not carried out on a statistical basis, it is possible to state that cultures which have been kept in continuous darkness and then exposed to alternating light and darkness, do not exhibit periodicity until after twenty-four hours have elapsed. The reason twenty-four hours are required under these conditions, rather than the twelve hours of the preceding two experiments with continuous light, is clear on analysis. At the end of a period of continuous darkness most of the sporangiophores are in stage II. When the culture is illuminated at (for example) 8 A.M., the sporangiophores which are in stage II begin to form sporangia, but also more sporangiophores continue to emerge from the agar. Thus at the end of twelve hours of light, sporangiophores are to be found in all stages of development, although most are in the maturation period. After the light has been discontinued at 8 P.M. the sporangiophores continue to emerge, but only until 10 P.M. As a result, at 8 A.M. the next morning (twenty-four hours following the first twelve-hour exposure to light) the sporangiophore count is similar to that of cultures which have been exposed to normal alternating light and dark periods.

No attempts have been made to determine what length of exposure to continuous darkness is sufficient to cause cultures growing in alternating light and darkness to lose their periodicity.

The observations and experiments just presented have given complete confirmation to the general belief that it is alternation of day and night which brings about daily formation and maturation of the fruiting bodies of *Pilobolus*. The experiments have demonstrated that periodicity of reproduction is not the expression of a behavior pattern fundamentally independent of the environment, but rather a characteristic reaction to a particular set of environmen-

tal stimuli. The problem thus becomes one of discovering how *Pilobolus* reacts as it does. What further advance the writer has been able to make has come from an analysis of the preceding seven experiments in terms of sporangiophore behavior within the agar.

OBSERVATIONS ON SPORANGIOPHORE BEHAVIOR WITHIN THE AGAR.—It will be recalled that in agar cultures of *Pilobolus* exposed to alternating light and darkness, the diurnal succession of crops of mature fruiting bodies was found to depend upon sporangiophore emergence from the agar during a definitely limited interval of time only. Cultures exposed to

the dark period began, and resumed growth seven or eight hours after the (following) light period had started. Sporangiophores growing within the agar in cultures exposed to continuous light or continuous darkness, on the other hand, did not exhibit this daily cessation and resumption of growth, but elongated through the agar at a steady rate, without forming any swellings.

These observations on sporangiophore behavior within the agar fully explain the time of sporangiophore emergence from the agar. In cultures exposed to normal alternating light and darkness sporangio-

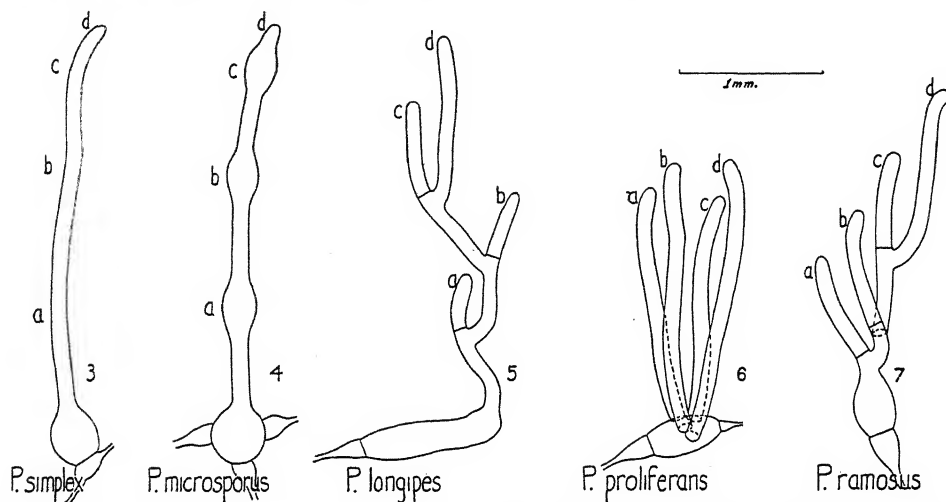


Fig. 3-7. Diagrams of different behavior patterns of sporangiophores growing within the agar, in *Pilobolus* cultures subjected to alternating twelve-hour periods of light and darkness.—Fig. 3. *P. simplex*.—Fig. 4. *P. microsporus*.—Fig. 5. *P. longipes*.—Fig. 6. *P. proliferans*.—Fig. 7. *P. ramosus*. In all figures, a-d represent the termination of the first, second, third, and fourth day's growth of the sporangiophore respectively.

continuous light or continuous darkness did not exhibit periodicity because the sporangiophores did not emerge from the agar rhythmically. The explanation of the phenomenon of periodic emergence from the agar was finally obtained from analysis of certain peculiar swellings regularly exhibited by sporangiophores of *P. microsporus* (fig. 4) when allowed to elongate through the agar for several days. Preliminary observation of sporangiophore growth under such conditions showed that the sporangiophore did not elongate through the agar continuously, but stopped growth during the evening. Next morning the sporangiophore swelled laterally at its apex (fig. 4a), and resumed forward growth in the afternoon. This behavior was repeated daily until the content of the trophocyst had become exhausted, or the sporangiophore had emerged from the agar. Since each swelling represented the termination of one day's growth, a count of the number of swellings (fig. 4a-d) gave the age, in days, of the sporangiophore.

When these observations were extended to cultures exposed to twelve-hour alternating periods of light and darkness, it was found that the sporangiophores stopped elongating about two hours after

phores emerge from the agar between 3 P.M. and 10 P.M., since the sporangiophores within the agar are elongating during that interval of time only. In cultures exposed to continuous light or continuous darkness sporangiophores emerge from the agar continuously because the sporangiophores within the agar elongate continuously.

Investigations of sporangiophore behavior among other species of *Pilobolus* have yielded interesting results. In all species examined, cultures exposed to alternating light and darkness showed daily cessation and resumption of growth of the sporangiophore within the agar. However, the manner in which this phenomenon is expressed varies among the species. An extreme divergence from the growth pattern of *P. microsporus* is seen in the sporangiophore behavior of *P. proliferans* sp. nov.² (fig. 6). In this spe-

² Since the writer's paper on the taxonomy of *Pilobolus* will not appear for several months, brief diagnoses (based on growth in nutrient agar culture) of three new species of this genus are appended here.

P. proliferans sp. nov. (fig. 6). Sporangiis lenticularibus v. ovoideis; columellis conicis; vesiculis subsporangialibus subellipsoideis; sporangiophoris intra substratum ex trophocystibus cotidie renovatis; trophocystibus ovato-cylind-

cies the sporangiophore which stops growing at 10 P.M. does not resume growth in the afternoon of the next day. Instead, the protoplasm withdraws completely from the sporangiophore, flows back into the trophocyst, and a septum is laid down at the base of the sporangiophore. Then at 3 P.M. a new sporangiophore is protruded from the trophocyst. If in turn this sporangiophore does not emerge from the agar by 10 P.M., the cycle is repeated. Trophocysts of this species have been seen with ten sporangiophores, representing as many days' growth.

A growth pattern which is intermediate between that of *P. microsporus* and *P. proliferens* is displayed by the sporangiophore of *P. longipes* van Tieghem (fig. 5). In this species the protoplasm withdraws only halfway down the sporangiophore, after the latter has stopped elongating. A transverse wall is laid down at this point, and at 3 P.M. the next day a new sporangiophore grows forth from the old, just proximal to the transverse septum. A modification of this type of sporangiophore growth pattern is found in *P. ramosus* sp. nov. (fig. 7) and also in *P. Kleinii* and *P. crystallinus*. In all three species the protoplasm withdraws a varying distance down the sporangiophore.

The sporangiophore of *P. simplex* sp. nov. (fig. 3) behaves almost precisely as that of *P. microsporus*: there is no retraction of protoplasm, no branching of the sporangiophore. However, in *P. simplex* no lateral swelling takes place after elongation for the day has ceased. Thus this growth pattern lacks any regularly recurring morphological peculiarity, and except by daily observation we are unable to determine how long a sporangiophore has been growing within the agar.

Among those species which possess branching sporangiophores a further significant phenomenon has been observed. After growth of the sporangiophore has ceased at 10 P.M., occasionally not all of the protoplasm withdraws toward the proximal end. The protoplasm which remains behind becomes walled off from the rest of the sporangiophore. In the afternoon of the following day a slender sporangiophore, much smaller in diameter than normal, grows forth from the walled-off remnant of protoplasm at the same time (3 P.M. to 5 P.M.) that the new sporangiophore is normally growing forth from the old. The slender "secondary" sporangiophore behaves precisely as the primary sporangiophore, branching characteristically with each day's growth.

draceis, uno apophyside; sporis ellipsoideis, decoloratis, hyalinis, $8.7 \times 6.3 \mu$.

P. ramosus sp. nov. (fig. 7). Sporangiis globosis v. subglobosis; columellis conicis; vesiculis subsporangialibus ovoideis v. piriformibus; sporangiophoris intra substratum ex sporangiophoris diei prioris cotidie renovatis; trophocystibus subconicis, uno apophyside; sporis ellipsoideis, pallido aurantiaco-viridibus, hyalinis, $10.1 \times 5.9 \mu$.

P. simplex sp. nov. (fig. 3). Sporangiis lenticularibus; columellis conicis; vesiculis subsporangialibus ovoideis; sporangiophoris intra substratum cotidie crescentibus, formas non mutantibus; trophocystibus ovoideis, uno apophyside; sporis ellipsoideis, obscure aurantiaco-viridibus, granulatis, $11.2 \times 5.9 \mu$.

Mention should be made of another phenomenon of possible significance for the problem of periodicity. In cultures of *P. Kleinii* exposed to alternating light and darkness, those sporangiophores which chance to emerge from the agar after 10 P.M. continue to grow for only a short while. After cessation of growth does take place, the protoplasm withdraws, and further activity is deferred as usual until the afternoon of the next day, when the new sporangiophore grows forth from the old. Maturation of the sporangiophore thus depends upon emergence of the sporangiophore from the agar before 10 P.M. The writer believes that in tables 1 and 2 the sporangiophores in group A, after the period of darkness, may be those sporangiophores which emerged from the agar after 10 P.M., and thus should not have been counted. (At the time the first and second experiments were conducted, this phenomenon had not yet been discovered.)

Occasional references to branching sporangiophores have appeared in the literature of *Pilobolus*. Léveillé (1826) described a species of *Pilobolus* possessing trophocysts which gave rise to three or more sporangiophores. Coemans (1861), Klein (1872), Grove (1884, 1934), and Zopf (1888) all noted the (infrequent) occurrence of branched sporangiophores in dung culture. Morini (1906) described as a new species, *P. Borzianus*, a form in which the trophocyst regularly protruded two to three sporangiophores. Of all these authors, Coemans alone attempted to relate such "abnormal" branched sporangiophores to an environmental influence. He had observed that all the sporangiophores of a dung culture of *Pilobolus* ceased to grow following the occasion of a violent storm, but on the following day branched to give rise to new sporangiophores. The sporangiophores undoubtedly stopped growth because of early darkness brought about by the storm. Although these workers regarded branching sporangiophores as abnormal, it would be more nearly correct to call such sporangiophores atypical.

DISCUSSION.—The investigations presented in this paper have resulted in clarification of the problem of periodicity of asexual reproduction in *Pilobolus*. The most important result has been definitive establishment of the fact that periodic reproduction is the result of reaction to the influence of a single external factor—light. Further analysis of this periodicity has shown that in cultures exposed to alternating light and darkness periodic production of mature fruiting bodies is the inevitable consequence of periodic emergence of the sporangiophore (periodic emergence from the trophocyst, in dung culture; periodic emergence from the agar, in agar culture).

To be sure, the fact that sporangiophores regularly emerge from the trophocyst only during the afternoon, has been pointed out by previous workers. However, these investigators apparently regarded periodic sporangiophore emergence from the trophocyst as but one of several periodic steps in the development of the fruiting body, rather than as the one

step responsible for periodicity. This misunderstanding of the true significance of sporangiophore emergence was a logical result of the then prevalent belief that trophocysts are produced periodically, and that periodicity accordingly originates with trophocyst formation. This may perhaps explain why even so careful a worker as Gräntz failed to notice that in cultures of *Pilobolus* subjected either to continuous light or to continuous darkness, the sporangiophores do not grow forth from the trophocyst periodically. However, it should be pointed out, in extenuation, that in their experiments with various conditions of illumination, Gräntz and others were concerned not with the possible effect on the time of sporangiophore emergence from the trophocyst, but with the effect on subsequent development.

Now that periodic emergence of the sporangiophore has been shown to be the *sine qua non* of periodic formation of mature fruiting bodies, experimental investigation of the problem of periodicity in *Pilobolus* can be confined to the phenomenon of periodic sporangiophore emergence. For such investigations agar culture of *Pilobolus* is greatly advantageous, since the phenomenon is repeated daily by each sporangiophore within the agar. Perhaps a more nearly complete understanding of periodicity in *Pilobolus* may result in some clarification of the mechanisms involved in similar biological rhythms.

SUMMARY

In nutrient agar, as on dung, *Pilobolus* exhibits a diurnally rhythmic asexual reproduction. The periodicity in the former type of culture is not so precise as in the latter.

The results of experiments in which agar cultures of *Pilobolus* were subjected to various conditions of illumination demonstrate that the reproductive pe-

riodicity of this fungus is not inherent (autonomic) but completely conditioned by the diurnal alternation of day and night. Maturation of sporangiophores, which normally takes place during the morning, can be made to occur at any hour of the day or night, by suitably adjusting the alternating twelve-hour periods of light and darkness. The periodicity can be made to disappear by subjecting cultures either to continuous light or to continuous darkness.

In agar cultures the sporangiophores arise from fruiting body primordia, or trophocysts, which are completely submerged within the substratum, unlike the condition in dung where trophocysts are formed just below or upon the surface of the substratum. In agar cultures subjected to alternating periods of light and darkness the sporangiophores which arise from the trophocysts exhibit a daily cessation and resumption of growth, so long as they remain within the agar. Only after emergence from the agar can the sporangiophores develop into mature fruiting bodies. Consequently, periodic production of mature fruiting bodies in *Pilobolus* is considered to be dependent upon periodic emergence of the sporangiophores: periodic emergence from the agar, in agar culture; periodic emergence from the trophocyst, in dung culture.

The results of certain experiments suggest that *Pilobolus* may be a microaerophilic organism.

Sporangiophores within the agar which exhibit daily cessation and resumption of growth, form definite growth patterns characteristic for each species.

Brief descriptions are given of three new species: *P. ramosus* sp. nov.; *P. proliferens* sp. nov.; and *P. simplex* sp. nov.

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PHOTOSYNTHESIS AND ABSORPTION IN BLUE RADIATION ¹

G. Richard Burns

IN EARLIER work with white pine seedlings (Burns, 1937, 1938), a purely arbitrary value called the primary absorption spectrum was determined for each of the various types of plants. The primary absorption spectrum was the incident radiation minus the reflected, minus the amount transmitted by an acetone solution of the pigments at about the same concentration as in the plant. The spectra of the reflection, of the transmission, and of the sources were made with wide slits. Granted a constant quantum yield at the wave lengths involved and assuming that only radiation thus primarily absorbed was photosynthetically effective, the relative amounts of photosynthesis in the various portions of the spectrum were calculated and found to agree, within a few per cent, with the experimentally determined values at wave lengths greater than 5,000 Å. To the extent of this rather surprising agreement, it was assumed that the primary absorption spectrum and the curve for photosynthesis at equal incident quanta intensity against wave length were the same within certain limits. However, between 4,000 Å and 5,000 Å, the amount of photosynthesis was found to be about one-half of that indicated by the primary absorption spectrum. In contrast to this low photosynthesis in pine, Hoover (1937, fig. 1) found a maximum in wheat within this region. The results of other investigators vary and will not be included, since Gabrielsen (1940) has given a general review of this work.

The main purpose of the experiments in this paper is to show that this difference in the behavior of pine and wheat in the blue region is due to differences in the two plants and not to errors or differences in the experimental methods. Since the same apparatus was also suitable for the partial investigation of several other problems, the results of these incomplete investigations, while not so conclusive, are also included.

In the study of photosynthesis, curves giving the amounts of photosynthesis at equal incident quanta intensity against wave length are of value only in so far as they yield information on photosynthesis at equal absorbed quanta. The approximate agreement of the curves (fig. 1) for wheat and pine at wave lengths longer than 5,000 Å, in spite of the obvious differences in color and absorption, shows that the corresponding curves on an absorbed quanta basis would differ. While accurate determinations of the fraction of incident radiation absorbed by a land plant under the conditions of these experiments is beyond our ability, such determinations as we have been able to make indicate that the amounts of photosynthesis in pine seedlings at 4,358 Å, 5,461 Å, and 5,780 Å are less than would be called for by the absorbed quanta when compared to other wave lengths. The divergence is greatest at 4,358 Å. In wheat this difference between the amount of photosynthesis and

absorption is not great enough to exceed the experimental errors of the determinations. It would appear that pine has more photosynthetically inactive absorption at these three wave lengths than wheat.

The primary absorption spectra of various wheat plants were also determined, and the results indicate that, in this plant, there is no agreement between the primary absorption spectra and the amount of photosynthesis.

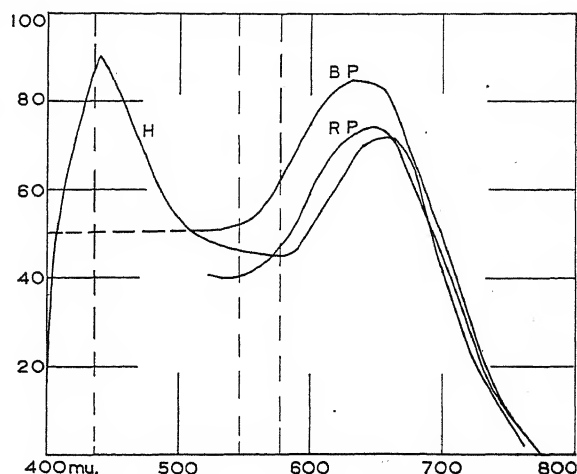


Fig. 1. H—Hoover's results with wheat graphed as photosynthesis at equal incident quanta intensity with maximum photosynthesis set at 90. BP—Curve for pine grown in blue light. The portion in dashes was covered in one experiment and indicates the average for these wave lengths. RP—Curve for pine grown in red light. The vertical dashes show the wave lengths studied in the present work.

APPARATUS AND METHOD.—The top of the plant was enclosed in a double walled, cylindrical glass container, 25 mm. in internal diameter and 120 mm. high, with distilled water from a bath at 27.0° circulating between walls. Large, polished Corning monochromatic filters were placed on either side of the container, at a distance of 20 mm., followed by 30 mm. water filters, and, finally, banks of four mercury lamps (type H 1, 400 watts). The plant was thus illuminated from two opposite sides by sources of considerable area. The light intensity was varied by turning off the middle two lamps in each bank and by moving the lamps. This last is an undesirable feature of the method, since moving the lights away from the plant decreases the angle at which light falls on the plant and makes more light, as measured by a flat surface thermopile, necessary to produce the same amount of photosynthesis. The error thus introduced was determined experimentally and found to be less than five per cent in extreme cases. The values given are corrected for this effect. The Corning filters transmitted less than one per cent of stray radiation in wave lengths that cause photosynthesis.

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Changes in the carbon dioxide concentration were determined by passing the air through 4 or 5 cc. of conductivity water surrounding a glass electrode and measuring the resulting voltage with a vacuum tube potentiometer. Since the experiment was designed to determine the light intensity at which photosynthesis balanced respiration, the sign of the change of carbon dioxide concentration and not its amount was determined. In order to obtain sufficient sensitivity with the glass electrode a carbon dioxide concentration of about 0.06 per cent was used, but with the rapid movement of air and the low light intensity this should not affect the results. With the amount of plant material employed, a 1 per cent increase in light intensity above the compensation point could be detected in five minutes. This system of determining changes in carbon dioxide concentration has the advantage of not withdrawing a sample of the gas, but it is very sensitive to temperature and pressure changes and to impurities in the water. The glass electrode system, the bellows air pump, and the water supply for the plant chamber were in an oil bath at $27.0 \pm .01^\circ$. The air was cooled between the glass electrode and pump to lower the humidity before returning it to the plant chamber.

In carrying out a determination, one or two days before the experiment the tops of the plants were sealed in the chamber by very soft grafting wax and illuminated with water-filtered radiation from an incandescent lamp for eighteen hours a day. The mercury lamps with one of the monochromatic filters were used to illuminate the plant for two or three hours before the actual determinations were begun. Readings of the carbon dioxide concentration were taken every five minutes, and the light intensity was changed by 10 per cent steps until the intensity was reached which kept the carbon dioxide concentration most nearly constant for four periods of five minutes each. The purpose of the four readings was to see whether or not the compensation point was shifting. Four readings were then made at a light intensity on the other side of the compensation point and the intensity at compensation determined by interpolation to the nearest five per cent. The light intensities were measured by a photronic cell. The filters were then changed, the plant illuminated for an hour in the new radiation, and the compensation point in this radiation determined. The last determination of the day was usually in the same radiation as the first, as a check against change in respiration or photosynthesis. After the plant had been removed, the light intensities were checked by a thermopile with a 25 mm. water cell. The results should be accurate to five per cent, since with this amount of preliminary lighting, the increase in efficiency, while usually noticeable, is much slowed down.

EXPERIMENTAL DATA AND DISCUSSION.—We have always found respiration to be practically constant over a period of eight hours if the plant has not been injured during installation in the reaction chamber. In some cases in which the compensation point was changing too rapidly to be measured, we have found

respiration to be changing only slightly. However, since Emerson (unpublished work) has found marked effects on respiration after illumination with certain wave lengths in the blue, measurements were made after illumination at 4,358 Å, but no changes greater than 2 per cent were found, which was the limit of accuracy of the glass electrode for this type of measurement.

The wheat plants used in this work were grown in a prepared soil that had been found to give a particularly lush growth and a much wider leaf than ordinary soil. The conditions of growth and the number and arrangement of the leaves were varied to make the primary absorption differ. Table 2 gives reflection and transmission values which are not absolute but are specific for the instrument with which they were made. The transmission value is particularly poor in cases in which more than one layer of leaves is involved, due to the difficulty of duplicating the register of the leaves, and does not adequately take into account variations in diffusion. A description of the plants used in this work follows:

1. Marquis wheat grown in artificial light, about fourteen days old. Single layer of leaves held flat toward light by wire frame and fine thread.
2. Wheat, artificial light, leaves random and same number as above.
3. Wheat, artificial light, triple layer of leaves held flat; before the second determination, the leaves, which were held too firmly at the top, had buckled out and filled the chamber. Slightly bleached at end of runs.
5. Wheat, artificial light, leaves random and same number as 3. Slightly bleached at end of runs.
6. Wheat grown in shade in greenhouse. Same size as above but about one month old. Single layer of leaves held flat.
7. Wheat, greenhouse, single layer held flat.
8. Wheat, greenhouse, triple layer held flat.
9. Wheat, greenhouse, triple layer held flat.
10. Wheat, grown under blue glass filter. Double layer of leaves held flat. Slightly bleached at end of runs.
11. Wheat, blue filter, mostly double layer but with some single layer.
12. Wheat, artificial light, single layer held flat.
20. White pine, grown in shade in greenhouse, new growth only.
21. White pine, greenhouse, new growth.
22. White pine, greenhouse, new growth.
23. White pine, greenhouse, lighter color, new growth.
30. Norway spruce grown under red glass filter.
31. Norway spruce grown under blue glass filter.
32. Norway spruce grown under blue glass filter.

In the tables and figures, Y is used to designate the 5,780 Å line; G, the 5,461; B, the 4,358; V, the 4,048; and UV, the 3,650. The light intensities are in arbitrary units based on incident calories. The efficiencies are based on incident quanta with the efficiency of the blue arbitrarily set at 90 for wheat and the green set at 50 for pine and spruce.

Photosynthesis in blue light.—Figure 2 and table 1 give the incident intensities of the different radiations that were necessary to keep the carbon dioxide constant. Figure 2 shows clearly that wheat is able to

TABLE 1. *The incident intensities of the yellow, green and blue mercury lines required to keep the carbon dioxide concentration constant with various plants. The relative efficiency is the photosynthesis at equal incident quanta intensity with the value for one wave length set at an arbitrary figure and the assumption of a constant quantum yield.*

Wave length	Intensity	Relative efficiency	Intensity	Relative efficiency	Intensity	Relative efficiency
Wheat 1			Wheat 2		Wheat 3	
Y	43	74	35	66	34	78
G	60	56	44	55	43	65
B	47	90	34	90	39	90
Y	42	74	30	77	38	77
G	61	54	42	58	59	52
B	46	90	34	90	43	90
Wheat 5			Wheat 6		Wheat 7	
Y	51	67	50	68	26	86
G	57	63	59	61	34	70
B	50	90	50	90	33	90
Y	51	67	—	—	32	66
G	57	63	60	59	39	57
B	50	90	49	90	31	90
Wheat 8			Wheat 9		Wheat 10	
Y	64	83	59	84	41	71
G	78	72	76	69	44	70
B	78	90	73	90	43	90
Y	47	75	56	74	45	74
G	52	72	59	74	50	70
B	52	90	61	90	49	90
Wheat 11			Wheat (Hoover)		White pine 20	
Y	51	82	—	50	56	57
G	63	71	—	50	68	50
B	62	90	—	90	79	54
Y	46	69	54	60
G	44	77	68	50
B	47	90	80	53
White pine 21			White pine 23		Norway spruce 30	
Y	63	67	56	68	43	48
G	89	50	80	50	44	50
B	111	50	98	51	51	54
Y	62	68	59	61	48	49
G	90	50	76	50	50	50
B	124	45	102	47	56	56
Norway spruce 30 (cont.)					Norway spruce 31	
Y	37	58			30	54
G	45	50			34	50
B	51	55			35	61
Y	36	51			28	57
G	39	50			34	50
B	45	55			35	61
Norway spruce 32					Norway spruce 32 (cont.)	
Y	30	49			28	52
G	31	50			31	50
B	42	46			39	50
Y	25	53			32	58
G	28	50			39	50
B	35	50			46	53

use incident blue light more efficiently than pine or spruce. It should be remembered that no attempt was made to determine these values to better than five

per cent. The actual intensities were used in this diagram, rather than the customary relative intensities, so that plants with the same photosynthetic be-

havior but with different amounts of respiration are separated and do not fall on the same points.

The variation between the results with the same plant on successive days is very noticeable and is probably due to three factors: a change in the register of the leaves, due to over-night growth, in the case of plants that had more than one layer of leaves held in a frame; an over-night change in respiration; and another unknown factor, more clearly shown in

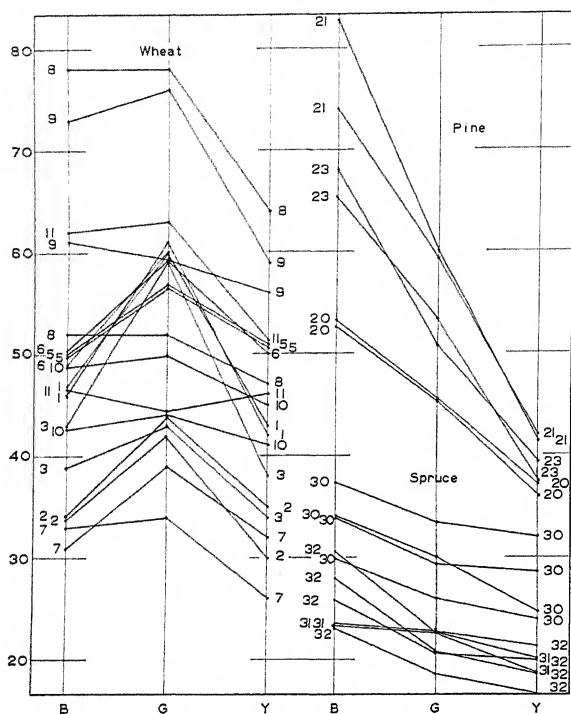


Fig. 2. Wave length and intensity of incident radiation required to keep the carbon dioxide concentration constant in the case of wheat, pine, and spruce. The scale of intensity for pine and spruce is two-thirds that for wheat. The purpose of the figure is to show that there is a difference in the behavior of pine and wheat in the blue.

earlier work in which photosynthesis was much higher than respiration, which sometimes causes a sudden increase in the amount of photosynthesis. Changing register of the leaves should affect the efficiency in blue radiation less than in the yellow or green, since blue radiation is more readily absorbed. This effect is best shown in plant 3, in which a triple layer of leaves was held flat the first day but buckled and filled the cylindrical container the second. Plants with a single layer of leaves (numbered 1, 6, and 7) and the plants with leaves distributed at random (numbered 2 and 5) should and do give more consistent results than plants with multiple layers of leaves (numbered 3, 8, 9, 10, and 11). The difference in the results with plant 8 may be due to a change in respiration.

Absorption and photosynthesis.—The absorption measurements (fig. 4) were made with a 175 mm. integrating sphere. A 10 by 20 mm. sample of the plant

was placed in the center and illuminated through a small opening in the front of the sphere. The phototube was placed inside the sphere facing the left side and 20 mm. from the center. Since the sphere was so small, it was tested by placing a flat magnesium oxide surface in the center and reflecting the radiation toward different portions of the interior. Reflection toward the entrance slit gave a reading of 100; to the right of the slit 96; left, 100; toward the right or left side, 99; toward the right rear, 103; left rear, 101. Reflection toward the left side threw most of the radiation on the shield which covered the back of the phototube. The inside of the sphere was coated with magnesium oxide from burning magnesium. The absorption measurements were not made on the

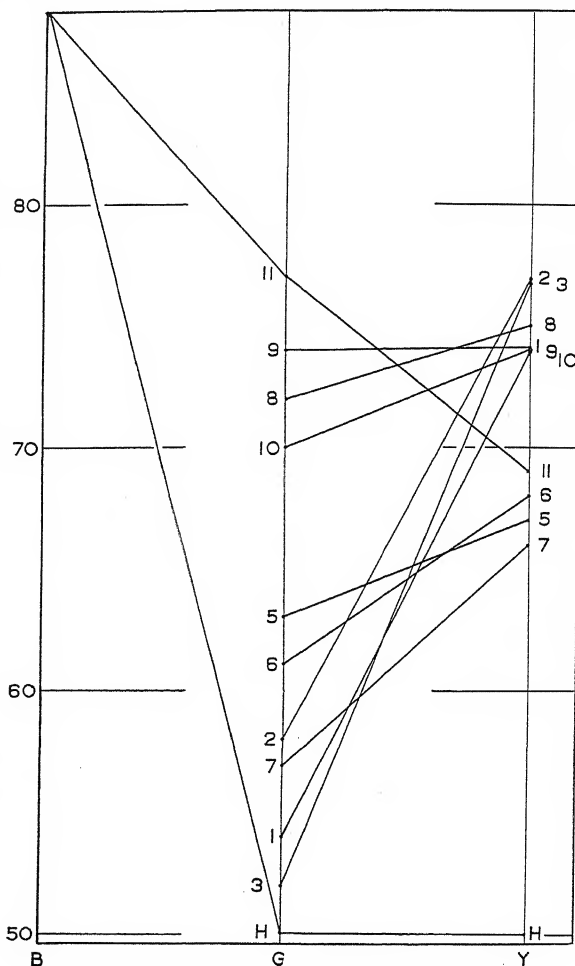


Fig. 3. Wave length and relative efficiency based on incident quanta for wheat with different pigment concentrations or different numbers of leaves in the reaction chamber. The efficiency of the blue radiation has been set at 90 in each case.

plants used in the experiments but on samples from similar plants. Since an appreciable amount of light passed between the needles in the measurements with pine, a correction was made for this by assuming

that all the blue radiation which was not reflected was absorbed and that the transmission in this case, 3.3 per cent of the radiation reflected by the magnesium oxide surface, was entirely due to radiation coming through cracks between the needles. Due to the different effects of line voltage variations on vapor and incandescent lamps, the measurements were made to 2 per cent.

The simplest concept of photosynthesis at different wave lengths would be to assume a constant quantum yield at all wave lengths capable of causing photosynthesis and to expect the amounts of photosynthesis to be proportional to the absorbed quanta. A comparison of the absorption values for wheat with the relative efficiency of the incident radiation (fig. 4 together with the additional efficiency values given in fig. 3) shows that this concept will apply to wheat, at the wave lengths studied, within the rather large experimental errors of the determination of these two values. In making this comparison it should be remembered that the efficiency of 90 in the blue is an assumption made necessary by the failure to find any suitable line source in the region of maximum absorption in the red. In view of Hoover's results, this assumption does not seem unreasonable. A group of lines around 7,000 Å may be isolated from the mercury lamps; these were tried in ten experiments with wheat and spruce, but the efficiency was about the same as in the yellow. Comparison of the absorption values for pine with the relative efficiency (fig. 4 and plants 20 to 24 in table 1) indicates that this simple concept does not hold for pine at any of these wave lengths. Apparently photosynthesis is far below absorption in the blue and well below it in the green and yellow. The efficiency of 50 for the green is assumed from previous work, and, if its validity is questioned, no definite conclusions can be drawn about photosynthesis in the green and yellow. Photosynthesis in the blue is so low, however, that there can be little doubt that it falls below absorption. Arithmetical comparison of photosynthesis and absorption would require absorption data from the actual plant used in the photosynthesis determination. These more accurate data would doubtless disclose differences between the absorption and photosynthesis in wheat, differences too small to be found by the present method.

Several things might be mentioned in regard to the individual efficiencies in figure 3. Only the figures for the second day are used, and with the plants numbered 7, 9, and 11 these differ widely from those on the first day. The results fall into three classes. The plants (numbered 8, 9, 10, and 11) with multiple layers of leaves and high pigment content had high relative efficiencies in the green and yellow. The plants with single layers of leaves and low pigment content (numbered 1, 2, and 3) had very low efficiency in the green and, from our point of view, too high efficiency in the yellow. The other plants (numbered 5, 6, and 7) with multiple layers of leaves of low pigment content or single layers of leaves of high pigment content fell between the other two

classes in the green and were lowest in the yellow. A fourth class would be Hoover's wheat with equal and lower efficiency in both the yellow and green. While none of these plants give the same results as Hoover's, there is enough variation between them to

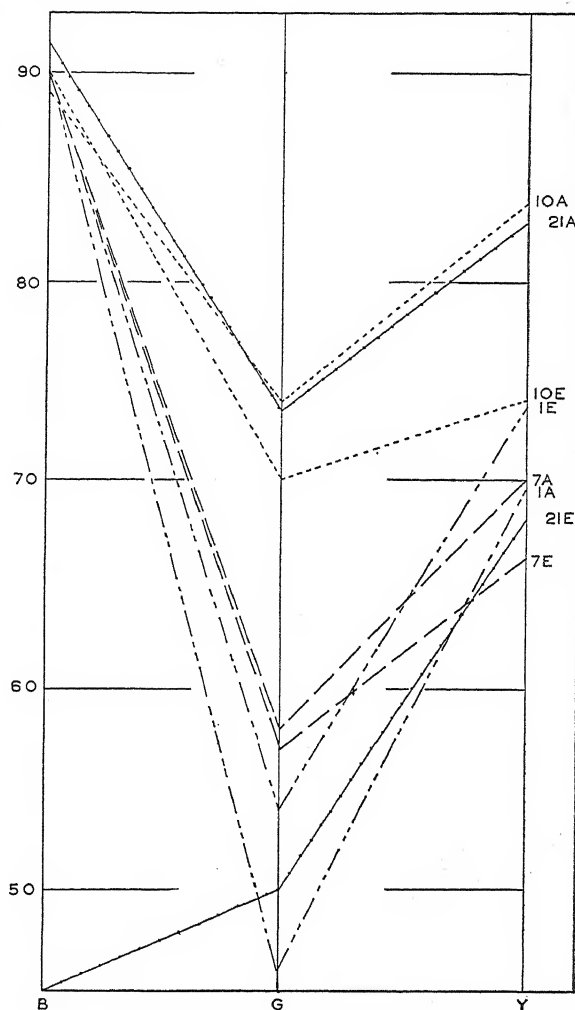


Fig. 4. A is the absorption and E is the efficiency based on incident quanta. 1 = Single layer of wheat grown in artificial light. 7 = Single layer of wheat grown in shade. 10 = Double layer of wheat grown in shade. 21 = Pine grown in shade. Absorption and efficiency correspond within the experimental error with wheat, but with pine the large divergence increases toward the shorter wave lengths. The absorption and efficiency were measured on similar, but not the same plants.

lead to the supposition that Hoover's results could have been duplicated had the plants been grown in the same manner. These results emphasize the point, perhaps not too apparent to those not engaged in this type of work, that any photosynthesis curve applies accurately only to plants grown under identical conditions.

Primary absorption.—The agreement between the primary absorption spectrum of pine and the

TABLE 2.

Plant	Wave length	Per cent reflection	Per cent transmission	Per cent transmission solution	Per cent primary absorption	Primary absorption factor	Relative efficiency
1	Y	20	50	60	32	2.31	74
	G	20	54	77	18	3.00	54
	B	15	17	1	84	1.07	90
2	Y	20	57	50	40	1.92	77
	G	28	63	70	22	2.63	58
	B	15	16	1	84	1.07	90
3	Y	25	23	54	35	2.20	77
	G	32	35	74	18	2.90	52
	B	21	5	0	79	1.14	90
5	Y	25	27	38	47	1.43	67
	G	30	42	54	32	1.98	63
	B	19	6	1	80	1.12	90
6	Y	13	48	55	39	1.74	68
	G	15	50	73	23	2.57	59
	B	12	19	1	87	1.03	90
7	Y	19	49	52	39	1.69	66
	G	21	57	73	21	2.71	57
	B	16	17	1	83	1.08	90
8	Y	17	5	23	64	1.17	75
	G	17	13	51	41	1.76	72
	B	15	1	0	85	1.06	90
9	Y	20	8	26	59	1.25	74
	G	23	15	50	39	1.90	74
	B	17	1	0	83	1.08	90
10	Y	19	9	28	58	1.28	74
	G	17	10	48	43	1.63	70
	B	14	1	1	85	1.06	90
11	Y	18	24	37	52	1.33	69
	G	17	27	60	33	2.33	77
	B	15	11	0	85	1.06	90
20	Y	12	38	20	70	.86	60
	G	12	43	42	51	.98	50
	B	9	38	0	91	.59	54
21	Y	12	60	25	66	1.03	68
	G	12	63	46	48	1.04	50
	B	9	55	1	90	.50	45

amounts of photosynthesis in the region between 5,200 Å and 6,700 Å would be impossible if photosynthesis paralleled absorption. The results in the green and yellow, while not conclusive, are closer to the primary absorption spectrum than to the absorption spectrum of the plant. On the other hand, photosynthesis in wheat has much higher values in the green and yellow than the primary absorption spectrum demands. The primary absorption and the factor by which it must be multiplied to give the amount of photosynthesis are given in table 2. Since the factor varies from 1 to 3 and the factor for the green is always larger than for the yellow, there is no agreement between the primary absorption spectrum and photosynthesis with wheat. The agreement with pine could be explained on the basis of absorption in the yellow and green by photosynthetically inactive pigment.

Violet and ultra-violet radiation.—Table 3 gives a few determinations in the violet and ultra-violet region of the spectrum. Since the radiation was not strong enough to have photosynthesis balance respiration, the experimental technique was changed. After the plant had been in darkness for about fifteen minutes, the voltage of the glass electrode was determined at the beginning and end of a further period of about fifteen minutes' duration. The carbon dioxide concentration was then returned to a low value, the plant was illuminated, and the increase in time for the voltage to pass between the two values was determined. This gave a preliminary lighting time of fifteen or more minutes, which was too short for accurate work. The violet radiation was very weak, and the glass electrode was in an air bath, so that the probable error in the efficiency with pine would be plus or minus 75, and with wheat somewhat less. The error for ultra-violet, with its higher

TABLE 3. *The relative efficiency for pine and wheat at the blue, violet and ultra-violet mercury lines.*

Plant	Wave length	Incident intensity	Increase in time	Calc. intensity	Relative efficiency
Pine 21	B	124			50
	V	12.6	1.40	44	150
	UV	47.0	1.08	630	17
Pine 22	B	133			50
	V	16.7	1.12	156	46
	UV	52.4	1.12	490	16
	V	13.7	1.07	210	34
	UV	52.4	1.02	2,600	0
Pine 23	B	100			50
	V	14.5	1.11	146	37
	UV	52.5	1.00	—	0
	V	14.0	1.27	66	82
	UV	52.5	.96	—	0
Wheat 12	Y	44			74
	V	12.5	1.30	54	86
	UV	45.0	2.01	89	58
	V	12.6	1.44	41	113
	UV	44.0	2.29	78	66
Wheat H	Y				50
	V				18
	UV				14

intensity, would be around twenty for pine and ten for wheat. It can readily be seen that pine has little, if any, photosynthesis in the violet at 4,047 Å and none in the ultra-violet at 3,650 Å. Wheat, on the other hand, shows considerable photosynthesis at both these lines. The total pigments in the plants, diluted to one-fifth the usual strength in 90 per cent methyl alcohol, gave the following transmissions: pine, B—37 per cent, V—54 per cent, UV—47 per cent; wheat, B—44 per cent, V—64 per cent, UV—68 per cent. After the chlorophylls and some other pigments had been removed by shaking with alcoholic potassium hydroxide, the remaining yellow pigments in methyl alcohol solutions had the following transmissions: pine, B—88 per cent, V—89 per cent, UV—88 per cent; wheat, B—91 per cent, V—92 per cent, UV—96 per cent.

SUMMARY

The relative photosynthetic efficiencies, based on incident quanta, of white pine, Norway spruce and Marquis wheat have been determined in monochromatic radiation at several wave lengths, and some rough measurements of the absorption and the primary absorption have been made at the same wave lengths. The relative efficiency of both pine and spruce at 5,461 Å and 5,780 Å is the same as in our previous work, and the efficiency at 4,358 Å is about the same as 5,461 Å. The efficiency of wheat is highest in the blue, lower in the yellow, and the same or still lower in the green. The actual values depend

upon the concentration of the pigments or the number of leaves in a given volume. At 3,650 Å wheat shows large amounts of photosynthesis and pine none. The efficiency of wheat in the blue, green, and yellow radiation is roughly the same as its absorption and is not the same as its primary absorption. The efficiency of pine in the green and yellow radiation is much closer to the primary absorption than to the actual absorption, and in the blue radiation the efficiency is much lower than absorption. It would appear that pine has absorption of radiation by inactive pigments in the blue and probably in the green and yellow.

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THE GROWTH AND METABOLISM OF OAT SEEDLINGS AFTER SEED EXPOSURE TO OXYGEN¹

Harry G. Albaum, John Donnelly and Seymour Korkes

IN THE course of experiments on the growth of oat coleoptiles after seed exposure to different oxygen concentrations, Albaum (1940) found that, after exposure to pure oxygen for periods of time ranging from about eight to twenty-four hours, seed subsequently grown in air failed to show any appreciable coleoptile growth as compared to the controls treated in a similar way with air. Oxygen consumption measurements on intact grains just after treatment, furthermore, showed that the rate of respiration was only 17 to 19 per cent lower in the oxygenated grains as compared to the aerated ones, while growth, as determined by measurements on final coleoptile length, was inhibited to the extent of 85 per cent.

Most of the experiments reported in the recent literature, with few exceptions, present the effects of oxygen treatment at tensions above atmospheric, and few of these treat of the effects on growth. Burk (1930) found that the respiration and growth of *Azotobacter* were inhibited when the organisms were grown in oxygen at atmospheric pressure. More recently, Bean (1941) reported that when *Pneumococcus*, Type I, was exposed to oxygen for twenty-four hours at a pressure of 900 mm. Hg., growth was completely inhibited. In the experiments described in the first paragraph, the oxygenation of the grains was done in water and at atmospheric pressure.

Several studies appear in the literature on the effect of oxygen on enzyme activity. Libbrecht and Massart (1937) found that oxygen under 5 atmospheres pressure completely inhibited the action of a succino-dehydrogenase preparation. The same kind of action was reported by Bohr and Bean (1940), who found that when oxygen at high barometric pressure (7.6 atm.) was bubbled through a succino-dehydrogenase preparation from pork heart for 2½ hours, a decrease in enzyme activity ranging from 9 to 50 per cent occurred. This effect was irreversible. Lehmann (1935) made the observation that at pH of less than 7.4 the oxygen utilization by a succinic acid-succinic dehydrogenase system was not inhibited by almost pure oxygen (713 mm. tension), while at pH greater than 7.4, the oxidation was inhibited. The toxic effect of the oxygen was exerted only in the presence of succinic acid and was irreversible.

Investigating the effect of pure oxygen on an entirely different kind of enzyme system, Kwan and Tung (1938) found that oxygen at atmospheric pressure slowed the rate of proteolysis in acid glycerol extracts from sheep liver.

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That a relationship exists between growth and respiration (under the control of different enzyme systems) has now been clearly established for the oat coleoptile (Bonner, 1933, 1933a, 1936; Commoner and Thimann, 1941; Albaum and Commoner, 1941). The present investigations were undertaken in an attempt to explain the inhibitions in the growth of oat seedlings due to exposures to oxygen in terms of an analysis of several known enzyme systems. The activity of three enzyme systems was investigated: cytochrome oxidase, catalase and dehydrogenase.

MATERIAL AND METHODS.—Grains of *Avena sativa* L. var. *Fulghum* similar to those used in earlier experiments were hulled and soaked in distilled water with either continual oxygenation or aeration for twenty-four hours at room temperature. After the treatment, the grains were planted in beakers in contact with moist filter paper, as described in an earlier paper (Kaiser and Albaum, 1939). The plants were then allowed to continue their growth in the dark at 27°C. At various intervals the coleoptiles were measured to the nearest millimeter under an orange safe-light. Coleoptile length was used as an index of growth. All figures reported are the averages of at least twenty plants.

The first experiments on enzyme activity were carried out with extracts which were prepared in the following way. Grains treated in the above manner were removed from the beakers after thirty hours (total age from time of soaking—fifty-four hours). The embryos were carefully dissected from fifty seedlings, ground in sand in 2 cc. of M/15 phosphate buffer (Na_2HPO_4 , KH_2PO_4), pH 7.4, and taken up in 3 cc. of additional buffer. After light centrifugation the supernatant fluid was decanted and stored in the ice chest overnight. In all cases where "extract" is spoken of, reference is made to the supernatant fluid after one day's storage in the ice chest.

Cytochrome oxidase activity was determined by measuring the oxygen consumption of extracts in the presence of paraphenylene diamine (.433 g./100 cc.) and cytochrome C in a Warburg respirometer at 26°C. The usual procedure was to use 0.4 cc. of extract, 1.0 cc. of paraphenylene diamine, 0.2 cc. of cytochrome C, and 0.4 cc. of M/15 phosphate buffer. The cytochrome C was prepared from beef heart by the method of Keilin and Hartree (1937).

Catalase activity was ascertained by measuring the oxygen evolved by the extract acting upon a given quantity of hydrogen peroxide in a Warburg respirometer. The kinetics of the reaction were studied, and the time required to decompose half the peroxide was calculated from the time curve. The shorter the half time, all other factors being constant, the greater the enzyme activity. The usual procedure involved the use of 0.2 cc. of extract, 0.2 cc.

of 3 per cent hydrogen peroxide, and 1.6 cc. of M/15 phosphate buffer.

Dehydrogenase activity was studied by the Thunberg methylene blue technique. The addition of succinate produced no change in decolorization time, so that it was not used in any of the experiments re-

ported on. What, therefore, was being measured was dehydrogenase acting upon endogenous substrate. The side arms of the Thunberg tubes contained 0.5 cc. of extract, while the main vessel contained 0.5 cc. of methylene blue (M/15 phosphate buffer, pH 7.4—3 parts, .0004 M methylene blue—4 parts), and 0.5 cc. of M/15 phosphate buffer. After the tubes were evacuated for three to five minutes, they were placed into a 37.5°C. incubator and allowed to come to temperature equilibrium. The contents of the side arm were then tipped in. In the early experiments the time for complete decolorization was used

as an index of enzyme activity. In later experiments, the kinetics of the decolorization reaction were studied with an Evelyn photoelectric colorimeter, and the time for half decolorization, read from the time curve, was used as a measure of rate. Experiments carried out at 26° gave similar results.

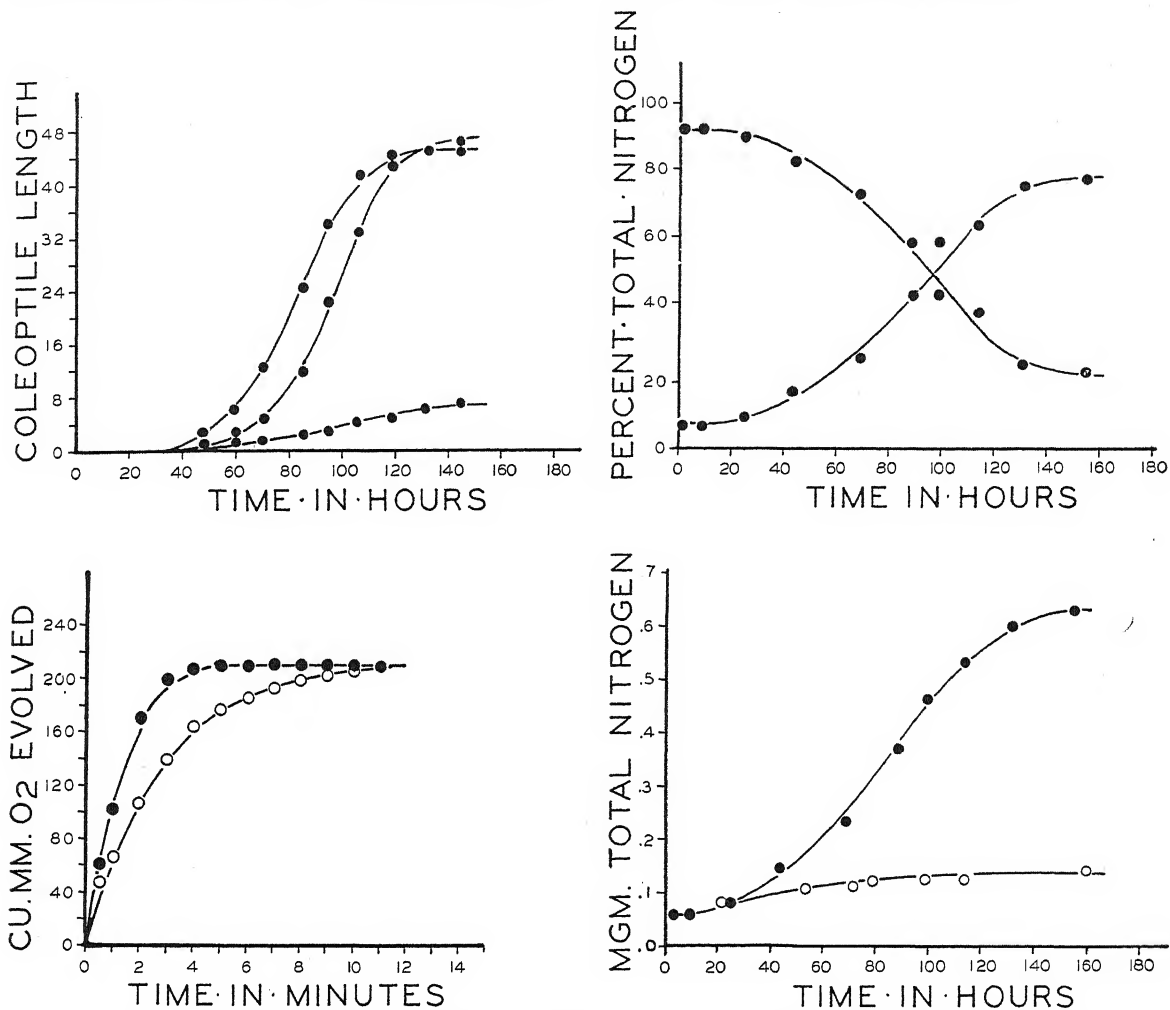


Fig. 1-4.—Fig. 1 (upper left). Growth of coleoptiles following seed treatment with air (upper curve), nitrogen (middle curve), and oxygen (lower curve).—Fig. 2 (lower left). Catalase activity of extracts from aerated seeds (closed circles) and oxygenated seeds (open circles).—Fig. 3 (upper right). Variation of total nitrogen content of endosperm (upper curve) and embryo (lower curve) with time.—Fig. 4 (lower right). Increase in total nitrogen in aerated embryos (closed circles) and oxygenated embryos (open circles) with time.

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In some of the experiments oxygen consumption measurements were made on intact embryos. These were also done in the Warburg respirometer and are recorded on a per embryo as well as on a dry weight basis. In order to carry out methylene blue decolorization experiments on intact embryos without interfering with the operation of the colorimeter, the following procedure was employed. Intact embryos were placed in the side arms of the Thunberg tubes. The methylene blue solution from the main vessel was tipped into the side arm, and the whole tube was kept in an inverted position during the course of the

run. Readings were taken at intervals by tipping the solution back into the main vessel and recording the decolorization in the colorimeter.

In the course of the investigations assays on total nitrogen were carried out. These were done by means of a micro-Kjeldahl technique in the usual way.

NaNO_2 is then tipped in and allowed to react with the contents of the main vessel for one hour. Then the KMnO_4 is tipped in and the flasks are shaken until all the NO_2 is absorbed. This is completed in about five minutes. The difference before adding the NaNO_2 and after adding the KMnO_4 multiplied by

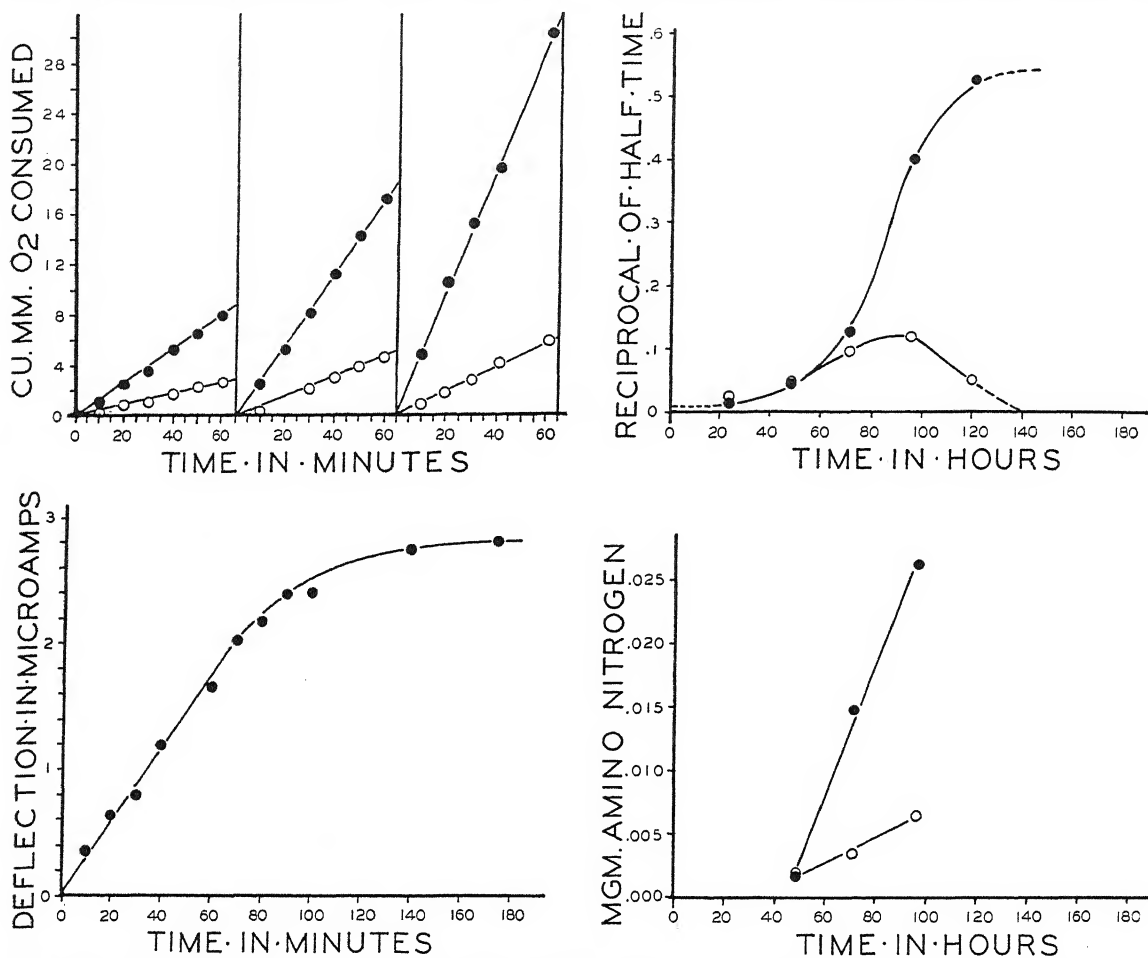


Fig. 5-8.—Fig. 5 (upper left). Oxygen consumption of intact embryos from aerated seeds (closed circles) and oxygenated seeds (open circles) at 48, 72 and 96 hours.—Fig. 6 (lower left). Decolorization of methylene blue as measured with the Evelyn photoelectric colorimeter by five intact control embryos.—Fig. 7 (upper right). Catalase activity of intact embryos from aerated seeds (closed circles) and oxygenated seeds (open circles) with time.—Fig. 8 (lower right). Increase in amino nitrogen in embryos from aerated seeds (closed circles) and oxygenated embryos (open circles) with time.

Amino nitrogen determinations were performed in the Warburg respirometer according to the method of Warburg, Christian and Griese (1935). In this method triple side arm flasks are used. Into the two connecting arms are placed 2 cc. of alkaline KMnO_4 (11 g. NaOH , 5 g. KMnO_4 , 89 cc. H_2O). The other side arm contains 0.4 cc. of NaNO_2 (9.8 g./100 cc.). Into the main compartment is placed the ground material to be tested in 1.8 cc. of water, together with 0.2 cc. of 33 per cent acetic acid. The vessels are then filled with nitrogen, transferred to the water bath at 26°C ., allowed to come to equilibrium without shaking and the manometers are read. The

the vessel constant and corrected for the blank (without the test material) is a measure of the amino nitrogen. One cu. mm. of gas evolved is equivalent to .000625 mg. of amino N.

EXPERIMENTAL.—GROWTH AFTER OXYGENATION AND AERATION.—The growth of *Avena* coleoptiles following exposure of grains during the soaking period to oxygen and air for twenty-four hours is shown in table 1 and figure 1. Also included are data on bubbling nitrogen through soaking grains in a similar way. Apart from an increase in the lag phase of the curve, the treatment with nitrogen produces no inhibition in growth. In fact, final coleoptile size is

TABLE 1. *Growth of grains of Avena sativa following soaking in distilled water for 24 hours through which air, oxygen or nitrogen has been bubbled.*

Time in hours	Coleoptile length in mm.		
	Air	Oxygen	Nitrogen
47.5	2.9	1.0	1.1
59.5	6.3	1.2	2.6
70.5	12.5	1.5	4.8
85.0	24.3	2.2	11.7
94.5	33.9	3.0	22.2
106.5	41.5	4.2	33.2
118.5	44.5	5.1	42.7
132.5	44.6	6.4	45.0
144.0	44.6	7.1	46.6

invariably larger after nitrogen treatment (for a discussion of this phenomenon, see Albaum, Kaiser and Eichel, 1940). The nitrogen treatment was carried out in order to rule out the possibility that the inhibition is due to the lack of CO₂ rather than to the presence of oxygen. In certain bacteria (Novak, 1908; Smith, 1924; Gladstone, Fildes and Richardson, 1935) carbon dioxide is necessary for growth. Bubbling carbon dioxide-free gas mixtures through culture media brings about growth inhibitions.

ENZYME ACTIVITY OF EXTRACTS.—Oxygen uptake of extracts.—The rates of oxygen consumption by extracts from treated and control embryos are presented in table 2 together with data on the addition

TABLE 2. *Oxygen consumption of extracts prepared from fifty embryos of aerated and oxygenated oat grains and sodium azide inhibition (extract—0.4 cc.; paraphenylene diamine [.433 g./100 cc.]—1.0 cc.; cytochrome C—0.2 cc.; M/15 phosphate buffer to final volume of 2.0 cc.). Temperature—26°C.*

	cu. mm. O ₂ /hr.
Aerated:	
Extract and buffer	4.8
Extract, paraphenylene diamine, cytochrome C	25.2
Extract, paraphenylene diamine, cytochrome C, sodium azide	17.6
Per cent inhibition due to azide	31.5
Oxygenated:	
Extract and buffer	26.6
Extract, paraphenylene diamine, cytochrome C	32.6
Extract, paraphenylene diamine, cytochrome C, sodium azide	20.5
Per cent inhibition due to azide	38.0

of cytochrome C, paraphenylene diamine and sodium azide. It will be noted that the endogenous oxygen consumption of normal extract is very low and is increased more than five fold by the addition of paraphenylene diamine and cytochrome C. The endogenous oxygen consumption of the extract from oxygenated embryos, however, is high and is stimulated only slightly by the addition of paraphenylene dia-

mine and cytochrome C. Several possibilities suggest themselves. Either the high endogenous respiration is due to a system other than the cytochrome oxidase system which might lead to a failure to respond appreciably to the addition of cytochrome C and paraphenylene diamine, or the high respiration is due to the presence of substrate which has not been used up. Consequently, the addition of paraphenylene diamine might not produce any large increase in oxygen uptake. That the latter interpretation is probably correct is borne out by what happens when azide is added. Sodium azide, according to Keilin (1936), specifically poisons the cytochrome oxidase. It will be noted that when azide is added the respiration of both the oxygenated extract and the aerated extract are inhibited to about the same extent and brought down to approximately the same level, suggesting the operation of the cytochrome oxidase system in both cases. It appears, therefore, that at least after thirty hours following treatment the cytochrome oxidase activity in aerated and oxygenated extracts is approximately the same; if anything, the activity of the oxygenated extract is slightly higher.

Catalase activity.—The results of a typical run on aerated and oxygenated extract in testing for catalase activity are given in figure 2 and table 3. It will

TABLE 3. *Catalase activity of extracts prepared from embryos of aerated and oxygenated oat grains (extract—0.2 cc.; H₂O₂ [3 per cent]—0.2 cc.; M/15 phosphate buffer—1.6 cc.).*

	Half time for peroxide decomposition in minutes	
	Aerated	Oxygenated
I	{ 0.9 0.9	1.9 2.1
II	{ 1.4 1.4	2.9 2.8

be noted that oxygen is evolved more rapidly by the aerated extract than by the oxygenated one. When half times are computed, it is apparent that the rate of peroxide breakdown is about twice as rapid in the control. There is then a clear cut difference in catalase activity following aeration and oxygenation.

Dehydrogenase activity.—The rates of methylene blue decolorization by treated and control extracts are shown in table 4. From this it is apparent that, just as in the case of catalase activity, oxygenated extracts show a lower dehydrogenase activity than aerated ones.

THE NITROGEN CONTENT OF SEEDLINGS.—The question now arises: does the oxygen treatment lead to the inactivation of enzyme already present in the embryo, or does oxygen prevent the formation of enzyme by the embryo? A study of the literature seems to indicate that in growing seedlings enzyme activity develops with time. Thus, Olcott and Thornton (1939) found that in cottonseeds the catalase content of whole seeds increased from 120 to 140

TABLE 4. *Dehydrogenase activity of extracts prepared from embryos of aerated and oxygenated oat grains (extract—0.5 cc.; methylene blue [M/15 phosphate buffer, pH 7.4—3 parts, .0004 M methylene blue—4 parts]—0.5 cc.; M/15 phosphate buffer—0.5 cc.).*

		Time in minutes to completely decolorize methylene blue	
		Aerated	Oxygenated
I	{	25	95
	{	25	95
	{	25	95
II	{	120	210
	{	120	210
	{	120	210

per cent in the first forty-eight hours of germination. Deleano, Popovici and Ionesco (1937) found that in wheat, oat and corn seedlings the catalase content increases for about the first ten days of growth, then decreases slightly and remains about constant. In germinating barley, von Euler, Myrbäch and Myrbäch (1930) reported that the catalase activity increases and reaches a maximum on about the eleventh day. The same is reported for proteinases in wheat by Mounfield (1936) and for peptidases in the soy bean by Stakheeva-Kaverzneva and Oleiknikova (1936).

Valy and Pokary (1939) made the significant observation that the catalase content of wheat is proportional, among other things, to the amount of nitrogen in the nutrient. In comparing extracts from experiment to experiment, the authors of this paper felt that they could best be compared in terms of total nitrogen, so that micro-Kjeldahl determinations were carried out on 0.2 cc. portions of extract routinely. In every case the total nitrogen of normal extracts was much higher than that of oxygenated extracts. In one typical case, 0.2 cc. of normal extract contained 0.236 mg. total nitrogen, while 0.2 cc. of oxygenated extract contained 0.047 mg. total nitrogen, or a five-fold difference. It was, therefore, decided to investigate the nitrogen content of embryos and endosperms as growth proceeds, in order to ascertain any differences which might arise following aeration and oxygenation and to correlate such results with the enzyme data.

Grains, weighing between 30 to 35 mg., were aerated and grown in beakers in the usual way. At

different intervals, plants were removed, embryo carefully separated from endosperm and total nitrogen determined for each. The results are shown in table 5 (upper part) and on figure 3 (expressed on

TABLE 5. *Total nitrogen content of aerated and oxygenated grains of *Avena sativa* at different stages of development. Each figure is the average of from six to twelve determinations.*

		Milligrams nitrogen		
Age in hours		Embryo	Endosperm	Whole seedling
Aerated				
0840
2	.058	.745		.803
9	.059	.720		.779
25	.080	.769		.849
43	.146	.709		.845
69	.233	.620		.853
88	.371	.511		.882
99	.465	.335		.800
114	.531	.316		.847
132	.600	.198		.798
155	.629	.193		.822
Average				.830
Oxygenated				
22	.085	.705		.790
53	.112	.738		.840
72	.116	.606		.722
79	.122	.630		.752
96	.123	.569		.692
103	.123	.675		.798
127	.123	.597		.720
160	.135	.622		.757
Average				.759

a per cent basis). It will be noted that the total nitrogen in the whole seedling remains relatively constant, but that with time the nitrogen content of the embryo increases at the expense of endosperm, suggesting that nitrogen moves from the endosperm to the embryo as growth proceeds.

When the same procedure is followed with seedlings from grains which have been oxygenated, one gets the data shown in table 5 (lower part). First it will be noted that the nitrogen content of the whole seedling remains fairly constant, as before, but that the figure is lower than that for aerated seeds. This

TABLE 6. *Oxygen consumption of intact embryos from aerated and oxygenated seeds.*

Age in hours		Cu. mm. O ₂ /hr./embryo	Dry weight of 10 embryos	Cu. mm. O ₂ /hr./g. dry weight
48	aerated	8.0	.0200 mg.	4,000
48	oxygenated	2.8	.0200	1,400
72	aerated	17.1	.0423	4,040
72	oxygenated	4.5	.0313	1,440
96	aerated	25.6	.0694	3,700
96	oxygenated	5.9	.0358	1,650

TABLE 7. *Dehydrogenase activity of intact embryos from aerated and oxygenated seeds.*

Age in hours		Half time for methylene blue decolorization in minutes for ten embryos
48	aerated	190
48	oxygenated	210
72	aerated	55
72	oxygenated	90
96	aerated	15
96	oxygenated	96

TABLE 8. *Amino nitrogen content of embryos and endosperms from aerated and oxygenated grains.*

Age in hours	Milligrams amino N/embryo or endosperm			
	I		II	
	Aerated		Oxygenated	
	Embryo	Endosperm	Embryo	Endosperm
48	.0017	.0232	.0019	.0128
72	.0149	.0218	.0034	.0161
96	.0262	.0299	.0065	.0154

difference may or may not be significant. What is more important, however, is that for a time the nitrogen content of the embryo increases slightly and then ceases to increase, while the endosperm loses a little nitrogen and then stops losing it. If the nitrogen data for aerated and oxygenated embryos are plotted together (fig. 4) and compared with the growth curves shown in figure 1, the similarity at once becomes apparent. There is a correlation, then, between the movement of nitrogen and growth. The same kind of nitrogen transport in normal plants is suggested by the work of Bonnet (1929) in *Lupinus*, by Echevin and Brunel (1937) also in *Lupinus*, and in *Lathyrus* by Isaac (1938).

THE DEVELOPMENT OF ENZYME SYSTEMS IN AERATED AND OXYGENATED EMBRYOS.—*Cytochrome oxidase activity*.—The oxygen consumption of extracts from oxygenated and aerated grains is not appreciably different at least up to four days of growth, when tested with paraphenylene diamine and cytochrome C. Yet when the oxygen uptake of intact embryos is measured in the Warburg respirometer, one obtains results shown in figure 5. The increase in oxygen uptake at 48, 72 and 96 hours in normal embryos is similar to the normal growth increase (see fig. 1). The increase in oxygen uptake in treated embryos also corresponds to the growth increase. There would appear to be a correlation between rate of oxygen uptake with time, growth and nitrogen transport. To test the possibility that the oxygenated embryos respire less because they are smaller, the above data have also been calculated on a dry weight basis. The data are shown in table 6. Even on a dry weight basis the aerated embryos respire more rapidly than oxygenated ones. It is also interesting to

note that the rate of oxygen uptake on a dry weight basis remains approximately the same at 48, 72 and 96 hours.

Dehydrogenase activity.—If it is true that the cytochrome oxidase activity of normal and treated extracts is about the same up to about four days, how is one to account for the differences in oxygen uptake by intact embryos during the same interval? The most likely possibility is that the oxygen uptake of the intact oxygenated embryos is lower because of lower dehydrogenase activity. A depression in the dehydrogenase end of the respiratory cycle will depress the activity of the oxidase end. That dehydrogenase activity is lower in oxygenated extracts has already been demonstrated (table 4).

In this portion of the work dehydrogenase activity was assayed on intact plants using the Evelyn photoelectric colorimeter to measure methylene blue decolorization. A typical run with five normal embryos is shown in figure 6. The time for half decolorization as read from the curve was used as an index of enzyme activity. The results with oxygenated and aerated embryos are summarized in table 7. It will be noted that the rate of decolorization is only slightly more rapid at 48 hours in aerated embryos, but that the rate changes abruptly at 72 and 96 hours. The rate of decolorization of oxygenated embryos increases, but not as much as the controls, and attains a maximum rate at about 72 hours, corresponding to the time at which nitrogen has ceased to move across from the endosperm. There appears to be a correlation thus far between growth, nitrogen transport, rate of oxygen uptake and dehydrogenase activity.

Catalase activity.—Catalase activity was assayed as before with extracts made at 24, 48, 72, 96 and 120 hours. The results are shown in figure 7. It will be noted again that there is a parallelism between growth, rate of oxygen consumption, dehydrogenase activity, nitrogen transport and now catalase activity. It also appears from figure 7 that up to about 96 hours the rate of enzyme development in oxygenated embryos is lower than that of normal embryos, but that at 120 hours there is no longer development but destruction of enzyme activity as well. From other experiments the same phenomenon is observed for dehydrogenase activity.

PROTEIN BREAKDOWN IN THE ENDOSPERM.—Thus far it appears that the chief effect of the oxygen treatment has been to produce some kind of irreversible change which manifests itself in the failure of most of the nitrogen to move from endosperm to embryo. Correlated with this failure of nitrogen to be transported is a corresponding slowing down in the rate of catalase and dehydrogenase production. All these in turn are correlated with the failure of plants treated in such a manner to grow normally.

What occurs in the endosperm to prevent nitrogen from moving across to the embryo? That the effect is probably not due to an elimination of CO₂ and a consequent upset in the carbonate buffer systems is indicated by the nitrogen growth curve in figure 1, where

CO₂ is presumably washed out as effectively as with oxygen. Apart from an increase in the lag phase of growth, plants treated in such a way attain at least normal size.

In the seed, between 85 and 90 per cent of the nitrogen is protein nitrogen (Bonnet, 1929; Isaac, 1938). Since the embryo contains only a small portion of the total nitrogen of the seed before growth occurs, it is safe to say that most of the protein occurs in the endosperm. It is also safe to say that, before the nitrogen can be transported from the endosperm to the embryo, the large protein molecule must be broken down to a more soluble form, i.e., amino or amide nitrogen. One might, therefore, expect to get an increase in amino nitrogen in the embryo at the same time that total nitrogen is decreasing in the endosperm. That this kind of phenomenon occurs is shown by the data in tables 5 (upper part) and 8 (I). It will be noted that as total nitrogen in the endosperm decreases, the amino nitrogen in the embryo increases. The amino nitrogen in the endosperm appears to remain constant, which probably means that an equilibrium in breakdown is maintained, the excess amino nitrogen being continually transferred to the embryo. In plants which have been oxygenated the total nitrogen in the endosperm decreases only slightly. In the embryo (table 8, II), there is less amino nitrogen, suggesting that protein breakdown in the endosperm is also occurring more slowly. This is borne out by the figures from oxygenated endosperms where there is always less amino nitrogen than in normal endosperms of the same age. It will be noted, too, that whereas the amino nitrogen in normal embryos increases more than fifteen fold between 48 and 96 hours, in oxygenated embryos the increase is only three fold. The data for amino nitrogen increase are shown graphically in figure 8. Again there appears to be a correlation between all the phenomena studied: growth, total nitrogen transport, rate of oxygen uptake, dehydrogenase activity, catalase activity and finally movement of amino nitrogen.

GENERAL DISCUSSION.—The data seem to indicate, then, that all of the phenomena noted may be due to the failure of the protein in the endosperm to break down. This would prevent nitrogen transport, which in turn may prevent the development of the different enzyme systems, and consequently prevent growth. The exact mechanism by which oxygen may produce its effect on nitrogen breakdown is not clear. It may be due to a direct effect on proteolysis (Kwan and Tung, 1938) or on the development of proteolytic enzyme activity in the endosperm.

Another possibility suggests itself. The protein breakdown may be prevented by the failure of the amino nitrogen to move across into the embryo. That is, proteolytic enzymes may be present in sufficient quantity, but there may be something wrong with the mechanism which accounts for moving the nitrogen through the cotyledon into the embryo. One might not expect the protein breakdown to occur continu-

ously unless the end products of the breakdown are removed.

That the phenomenon may not be due simply to the failure of nitrogen to move from endosperm to embryo is suggested by some preliminary results which indicate that auxin also is not transported when grains have been treated with oxygen.

Additional experiments to elucidate further the phenomenon are now under way.

SUMMARY

When grains of *Avena sativa* are oxygenated during the soaking period, subsequent growth is almost completely inhibited as compared to aerated controls.

Enzyme assays carried out on extracts thirty hours after oxygenation indicate little or no change in cytochrome oxidase activity, a lowered catalase activity and lower endogenous dehydrogenase activity.

In the course of normal development, nitrogen moves from endosperm to embryo. The increase in embryo nitrogen follows the course of growth. In plants grown from oxygenated grains, nitrogen increase in the embryo occurs more slowly for a time and then stops. This behavior is correlated with the same type of growth pattern.

In the case of catalase it is shown that oxygenation at first slows down catalase activity and later causes catalase destruction.

The development of catalase and dehydrogenase activity in intact embryos seems to be correlated with the movement of nitrogen from the endosperm to the embryo in both normal and oxygenated grains.

The failure of nitrogen to move from endosperm to embryo in oxygenated plants does not appear to be due to the mechanical removal of carbon dioxide by pure oxygen, since nitrogen gas produces no comparable inhibition in growth.

In plants grown from oxygenated plants, amino nitrogen is lower in embryo and endosperm, suggesting that oxygen may interfere with proteolytic breakdown in the endosperm and, consequently, prevent nitrogen transport, the development of enzyme activity and finally growth.

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LONGEVITY OF SUGARCANE AND CORN POLLEN—A METHOD FOR LONG-DISTANCE SHIPMENT OF SUGARCANE POLLEN BY AIRPLANE¹

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A study of pollen longevity and the means of extending it is important not only because it makes possible the shipment of pollen over great distances, so that plants separated by thousands of miles may be crossed, but it also makes it possible to bridge over long intervals separating the flowering of different forms which one may desire to cross. The purpose of this study was to find a method of storing and packing sugarcane pollen for long-distance shipments by air in order to cross a cold-resistant, Temperate Zone wild sugarcane, *Saccharum spontaneum* L., which does not flower in the Tropics, with the large cultivated tropical sugarcane.

The wild cane was growing at Arlington Experiment Farm, Rosslyn, Virginia, near Washington, D. C., and flowered during July and August (Brandes *et al.*, 1939). Since cultivated sugarcanes in the Northern Hemisphere flower during late autumn and winter, it was necessary, in order to make the cross, to ship the pollen of the wild cane to the

Southern Hemisphere where the seasons are reversed. The first successful pollen shipments were made during the summer of 1938. In addition, Brandes (1939) successfully transported living plants of the wild cane in the early flowering stage from Washington, D. C., to Colombia, South America, by steamer.

—Andronescu (1915), Anthony and Harlan (1920), Knowlton (1922), Holman and Brubaker (1926), Kempton (1936), and Bair and Loomis (1941) have shown that the pollen of Gramineae has a very short life span. Under the normal conditions of temperature and humidity existing at the time of flowering, the pollen remains viable for only a few hours. Vijayasarahy (1939) kept sugarcane pollen viable for two days in a thermos bottle charged with ice and water. At the end of two days a suitable recharging was needed. There is general agreement among investigators that death of the pollen of grasses is due to loss of water and that duration of

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life is longer at fairly low temperatures and high relative humidities.

Failure of germination of the pollen of Gramineae on artificial media is a common difficulty. Apparently the water relations are very delicate, and the concentration has to be exactly correct before germination takes place. Venkatraman (1922) working with sugarcane pollen obtained the best germination on a medium of 0.7 per cent agar with 26 per cent sucrose. Knowlton (1922) obtained good germination of corn pollen on 0.7 to 1 per cent agar plus 15 per cent sucrose. The results were not constant. At times germination from a certain concentration was excellent, and on repetition the same strength of solution failed to induce germination. Bair and Loomis (1941) obtained as much as 90 per cent germination of corn pollen on a solution containing 0.7 per cent agar and 15 per cent sucrose at a temperature of 23°C. and 90 per cent relative humidity. With different lots of highly viable pollen the sucrose percentage showing maximum germination varied between 10 and 15 per cent. They stress the importance of sowing the pollen as soon as the medium has solidified. Best results were obtained when the pollen grains were two-thirds embedded in the medium. Germination improved with storage for about six hours, then declined rapidly. However, one lot of pollen was viable after ten days' storage at 8°C. Longer storage life was obtained at higher humidities, but many lots stored in nearly saturated air suddenly became moist and clumped together and thereafter showed no germination.

MATERIALS AND METHODS.—For experimentation at different temperatures the pollen was stored in a constant temperature chamber adjusted to 15°C. This temperature was controlled to within $\pm 0.5^\circ\text{C}$. Household electric refrigerators were used to obtain the storage temperatures of 7°C. and 4°C. Since the refrigerators were opened only once a day, when the pollen was tested for germination, the temperatures were controlled to within 1°C. The relative humidities were obtained by using saturated solutions of different salts. It was assumed that the relative humidity over water was 100 per cent. Sulphuric acid solutions were tried in a few experiments, but germination was adversely affected even at the lower concentrations (higher humidities).

To obtain the sugarcane pollen that was used in the experiments several tassels were gathered late in the afternoon and taken to the laboratory, where the stems were placed in a solution of 0.2 per cent sulphurous acid and 0.2 per cent ortho phosphoric acid, and the tassels were inclined over a large piece of plate glass. The following morning the tassels were tapped, and the pollen settled on the glass. The pollen was then swept up and screened through a 50-mesh copper screen to remove the anthers and stigmas that had fallen with the pollen. The few stigmas that remained were removed by placing the pollen on a sheet of stiff paper, inclining the sheet and tapping it lightly. The stigmas remained behind, while the pollen was gathered on another sheet of

paper. Ten or twelve tassels yielded from 15 to 30 grams of pollen for three or four successive mornings. Golden Cross Bantam corn (*Zea Mays*) pollen was gathered early in the morning from a field on Arlington Experiment Farm.

Weighed amounts of pollen were placed immediately in small chambers, which were maintained at different relative humidities, and stored in the refrigerator. The chambers were glass stoppered bottles of about 300 ml. capacity, in each of which there were 80 ml. of a saturated salt solution, a different solution for each degree of humidity. The pollen was placed in shallow vials which were suspended from the stopper. Other small chambers were made from test tubes. The shallow vials containing the pollen were suspended in the test tube by linen threads, and the threads were held securely in place by the rubber stopper used to close the tube. The germination of the stored pollen was tested each morning.

The viability of the sugarcane pollen was tested by germination on artificial medium composed of 1.5 per cent agar and 25 per cent sucrose, and that of the corn pollen on 1.5 per cent agar plus 18 per cent sucrose. Various concentrations of sugars from 10 to 45 per cent were tried, but the above concentrations gave the maximum germination for the cold-resistant wild sugarcane and Golden Cross Bantam corn, respectively. The agar was cooled to about 60°C. and poured into Petri plates or a layer about 1 mm. thick was spread on microscopic slides. Both the Petri plates and the slides gave satisfactory results, but the slides were more convenient to use. As soon as the agar jelled the pollen was dusted on from a scalpel. The slide was then held in a horizontal position and the edge tapped sharply with a pencil to break up lumps and to spread the pollen evenly over the surface of the agar. The slides were placed in a moist chamber which was only moist enough to prevent condensation on the slides. Germination at room temperatures, which ranged from 22° to 30°C., began in two to three minutes. Growth of the pollen tubes was very rapid, attaining a length of 200 to 400 microns in twenty to thirty minutes.

When artificial media are used for germination of sugarcane and corn pollen there should be no free moisture on the surface of the agar. When Petri plates were used, they were left uncovered until the agar had set and cooled to room temperature, so that there was no condensation when the plates were covered. Condensation water on the surface of the agar causes the pollen grains to burst.

RESULTS AND DISCUSSION.—The investigation of pollen storage at 7°C. consisted only of a study of the effect of temperature on the pollen longevity. Several experiments were made with the pollen from plants of the clone 59 Amu Darya (*S. spontaneum*). The pollen was placed in vials closed with cotton plugs. There was some variability in the length of time the pollen remained viable, but in all experiments there was good germination at the end of seven days' storage, and in two experiments a few grains germinated after storage for ten days. After

TABLE 1. Germination of sugarcane pollen (U. S. 4523) stored at 4°C. and at relative humidities from 39 to 100 per cent, August 22, 1940.

Relative humidity, per cent	Duration of storage days										Condition of pollen
	1	2	3	4	5	6	7	8	9	10	
Storage at 4°C.											
Over CaCl ₂	Good ^a	Fair ^b	None								Dry powder
39	Good	Fair	None	None	None						Dry powder
47	Good	Fair	None	None	None						Dry powder
52	Good	Fair	None	None	None						Dry powder
70	Good	Good	Fair	Fair	None	None	None				Grainy
76	Good	Good	Good	Good	Fair	None	None				Grainy
84	Good	Good	Good	Good	Fair	Very poor ^c	None	None	None		Lumps
90	Good	Good	Good	Good	Fair	Fair	Good	Fair	None	None	Moist
100	Good	Good	Good	Good	Fair	Fair	Good	Good	Poor ^d	Very poor	Dough
Control ^e	Good	Good	Good	Good	Fair	Poor	Poor	None	None	None	Moist
Room Temperature											
Over CaCl ₂	None	None	None								Dry powder
52	None	None	None								Dry powder
76	None	None	None								Lumps
100	Good	Fair	None	None							Dough
Control ^e	None										Moist

^a Good = 70 to 90 per cent. Germ tube grew four to five times diameter of pollen in twenty minutes.

^b Fair = 40 to 70 per cent. Germ tube grew four to five times diameter of pollen in twenty minutes.

^c Very poor = 10 per cent or less. Tube short, growth slow.

^d Poor = 20 to 40 per cent. Germ tube grew four to five times diameter of pollen in twenty minutes.

^e Pollen was in a cotton plugged vial, and humidity was not controlled.

three or four days' storage the pollen in the vials was moist.

The control pollen stored in cotton plugged vials at room temperature remained viable for twelve to twenty-four hours. Pollen that was spread out on a watch crystal in diffuse light in the laboratory remained viable for about six hours.

The results of the experiment at 15°C. and at relative humidities of from 39 to 100 per cent showed that this temperature was not favorable for prolongation of the life span. Pollen stored at 100 per cent relative humidity remained viable for three days, at 70 to 90 per cent for two days, and at humidities below 70 for one day.

The results of an experiment with the pollen of clone U. S. 4523 (*Saccharum spontaneum*), stored at a temperature of 4°C. and at relative humidities from 39 to 100 per cent, are given in table 1. Pollen stored at relative humidities of 39 to 52 per cent or over calcium chloride remained viable for one to two days; at 70 to 84 per cent the life duration was from four to six days, and at 90 to 100 per cent from eight to ten days, as indicated by the ability of the pollen to germinate on an artificial medium. Although high relative humidities increased the life span, the pollen became moist, in which state it was difficult to use for pollinating sugarcane flowers. The pollen stored at 90 per cent relative humidity was moist, and at 100 per cent it was so moist that it formed a stiff dough. The pollen in this state is a favorable medium for the development of fungi, and even at 4°C. after several days' storage there may be a considerable growth of mycelium penetrating the mass of pollen. At relative humidities of 70 to 84 per cent

the pollen has the appearance of coarse corn meal, owing to pollen grains adhering together to form clumps. However, even after storage for from six to eight days about half of the pollen was still dry and in a satisfactory physical state for making pollinations. Pollen stored at 50 per cent relative humidity or lower was a dry powder, but under such conditions it retained viability for a very short period. The pollen marked "control" was in a cotton plugged vial. Although the humidity of the refrigerator varied between 50 and 60 per cent, the humidity in the vial was much greater, as indicated by the pollen becoming moist. Since the longevity and the moisture of the control pollen was about the same as pollen stored at 90 per cent humidity, the relative humidity in the vial was about 90 per cent.

The pollen stored at room temperatures, which during July and August ranged from 22° to 35°C., had a short life span. At a relative humidity of 100 per cent the pollen was viable at the end of the second day of storage, and at lower humidities the life span was less than one day.

The results of experiments with sugarcane pollen stored in vacuum at 4°C. were extremely variable. When the pollen occupied one-half or more of the volume of sealed glass tubes there was no germination after storage for twenty-four hours. The moisture excreted by the pollen turned it into a doughy mass. When a small amount of pollen was sealed in a tube, there was good germination after two days' storage and a few grains germinated after four days.

The best results were obtained by using a large tube, a relatively small amount of pollen, and calcium chloride to absorb part of the water excreted by

the pollen. A bulb was blown at the end of a tube about 30 cm. long and 20 cm. diameter, and 2 grams of CaCl_2 were put in the bulb. The tube was bent at the middle to a right angle, and a bulb was blown near the open end. The open end was drawn down to a diameter of about 2 to 3 mm., and 2 gms. of pollen were placed in the empty bulb. After evacuating with a high-vac pump for thirty minutes, the tube was sealed. Two tubes were stored at 4°C . and one at room temperature. The pollen stored at room temperature failed to germinate when tested after two days' storage. One of the tubes stored at 4°C . was opened after four days' storage and the germination was as good as fresh pollen. The other tube was opened after eight days' storage and gave a germination of about 25 per cent, but growth was slow, so that only short pollen tubes were formed in twenty minutes. The experiments indicate that low pressure is not as favorable for storage as normal atmospheric pressure.

The behavior of the corn pollen was very similar to that of sugarcane pollen stored under the same conditions. The results of an experiment with the pollen of Golden Cross Bantam corn stored at a temperature of 4°C . and at relative humidities from 39 to 100 per cent are given in table 2. The life of the corn pollen was prolonged by low temperature and high humidity. At 90 and 100 per cent relative humidities the pollen was viable after ten days' storage. For the first six or seven days the germination and growth of the tubes of the stored pollen was as good as that of fresh pollen, but thereafter the percentage of germination dropped very rapidly so that, after seven or eight days of storage, less than 25 per cent of the pollen germinated, and growth of the pollen tube was very slow.

The results of these experiments show that the pollen of sugarcane and corn remained viable longest

under conditions of low temperature and high humidity. When sugarcane and corn pollen are exposed to normal atmospheric conditions or to low humidity, the degree of drying determines the duration of life. An excessive loss of water by the pollen is accompanied by a change in the physical state of the protoplasm. When pollen that had been stored for a day or longer at low humidities or over calcium chloride was placed on an agar medium, favorable for germination, the contents of the grains oozed out through the germ pores. This was not like the rapid movement or violent eruption of fresh pollen on a hypotonic medium. The slow oozing of coarse granular protoplasm a few seconds after the pollen was placed on agar indicates that some destructive change in the protoplasm has taken place. Nevertheless, the pollen can resist desiccation to a considerable degree without loss of vitality, as shown by storage at humidities of 70 to 84 per cent.

METHOD OF PACKING SUGARCANE POLLEN FOR LONG-DISTANCE SHIPMENTS BY AIRPLANE.—While the pollen retained its viability longest at a low temperature and high humidity, it became moist and caked, and this condition interfered with the mechanical operations of pollination. It was apparent, therefore, that it would be necessary to devise a method of packing that would keep the pollen viable and in about the same physical condition as fresh pollen. The storage experiments at humidities of 70 to 84 per cent showed that the pollen could resist considerable loss of moisture without loss of viability for four days, and that the pollen was dry enough for making pollinations; therefore, in the method adopted, calcium chloride was used to remove part of the moisture.

The pollen that was intended for shipment by airplane was gathered at about 9 A.M. from tassels that had been placed in the laboratory the night be-

TABLE 2. Germination of corn pollen stored at 4°C . and at relative humidities from 39 to 100 per cent.

Relative humidity, per cent	Duration of storage days										Condition of pollen	
	1	2	3	4	5	6	7	8	9	10		
Storage at 4°C.												
39	Good ^a	Fair ^b	None	None	None							Dry powder
47	Good	Good	Fair	None	None	None						Dry powder
52	Good	Fair	Fair	None	None	None						Dry powder
70	Good	Fair	Fair	Poor ^c	Poor	None	None	None	None			Grainy
76	Good	Good	Fair	Fair	Poor	Poor	Very poor	None	None	None	None	Grainy
84	Good	Good	Good	Good	Fair	Very poor ^d	Poor	Very poor	None	None	None	Lumps
90	Good	Good	Good	Good	Fair	Good	Fair	Fair	Poor	Poor	Poor	Moist
100	Good	Good	Good	Good	Good	Good	Good	Fair	Poor	Poor	Poor	Dough
Control ^e	Good	Good	Good	Good	Fair	Fair	Poor	Poor	Fair	Poor	Poor	Moist
Room Temperature												
Over CaCl ₂	None											Dry powder
100	Poor	None										Dough
Control ^e	Fair											Moist

^a Good = 70 to 90 per cent. Germ tube grew 3 to 4 times diameter of pollen.

^b Fair = 40 to 70 per cent. Germ tube grew 3 to 4 times diameter of pollen.

^c Poor = 20 to 40 per cent. Germ tube grew 3 to 4 times diameter of pollen.

^d Very poor = 10 per cent or less. Tubes short, growth slow.

^e The control was in a cotton plugged vial and humidity was not controlled.

fore. Vials of about 10 ml. capacity were filled half full with pollen, plugged with cotton, and dried in a desiccator over CaCl_2 for about two hours. Meanwhile, about 8 gms. of CaCl_2 were placed in a test tube (18 by 2.5 cm.), and the tube was closed with a rubber stopper. After drying for two hours, the vials containing the pollen were wrapped in cotton and placed in the test tube. The test tube was closed with a rubber stopper, placed in a precooled, wide-mouthed thermos bottle, and surrounded with moist peat that had been frozen. The thermos bottle was surrounded with ice caps and sections of automobile inner tubes filled with crushed ice. This was packed in a double-walled corrugated paper box of sufficient capacity so that the ice caps and thermos bottle were surrounded by about four inches of granulated cork. There were about six pounds of ice in each shipment. The ice caps were used because the shipments were by air express, and it was necessary that the ice be placed in watertight containers.

Solid carbon dioxide was tried, but it was not possible to control the temperature, and, at the temperature required, "dry ice" is not as efficient as ice.

On arrival at Palmira, Colombia, three days after packing, the vials of pollen were removed from the test tube and immediately placed in a refrigerator at 4° to 5°C . It was too late in the day to use the pollen on the date of arrival; therefore, pollinations were made the following morning and for two or three more successive mornings.

During the summer of 1939 eight shipments of pollen seedlings of 59 Amu Darya and 60 Amu Darya were made. These two clones of cold resistant *Saccharum spontaneum* are practically indistinguishable. However, 59 Amu Darya has 60 somatic chromosomes and 60 Amu Darya has 50 somatic chromosomes.

The pollen was used in crosses with P. O. J. 2725, a tropical commercial cane, and an abundance of fertile hybrid seed was obtained. Altogether the eight crosses made with the pollen shipped by air and the seven crosses made with the flowering plants of U. S. 4513 taken to Colombia by steamer produced more than 15,000 hybrid seedlings. The crosses made with the stored pollen produced as much viable seed as those made with fresh pollen from the plants of U. S. 4513. In August, 1940, two shipments of pollen of U. S. 4536, a selfed seedling of 59 Amu Darya, were sent to Colombia by air and used to pollinate P. O. J. 2725. About 3,000 hybrid seedlings were obtained. The ability of sugarcane pollen stored from four to

seven days to fertilize shows that germination on an artificial medium is a test of the ability of sugarcane pollen to function normally.

The hybrids are intermediate in stalk diameter, leaf width, and time of flowering. The male parent flowers in June and July in Louisiana, and a few flowers have been produced at the U. S. Sugar Plant Field Station, Canal Point, Florida, in June. At this station, however, growth is not normal, and it is only occasionally that some of the plants flower. The female parent at Canal Point, Florida, flowers in November–December. At Canal Point most of the hybrids flowered during August and September. In sucrose content and winter hardiness the hybrids tend more towards P. O. J. 2725.

SUMMARY

The maximum germination of the pollen of cold-resistant clones of *Saccharum spontaneum* was obtained on a medium composed of 1.5 per cent agar and 25 per cent sucrose.

The maximum germination of Golden Cross Bantam corn, *Zea Mays*, pollen was on a medium of 1.5 per cent agar and 18 per cent sucrose.

At temperatures from 22° to 30°C ., 80 to 90 per cent of fresh pollen germinated. Germination began in two or three minutes and growth of the pollen tube was very rapid.

Other conditions necessary for germination are that there must not be any free water on the surface of the medium, that the pollen be sown as soon as the agar gels, and that the cultures be kept in a moist atmosphere to avoid drying of the pollen.

Sugarcane and corn pollen remained viable for ten days when stored at 4°C . and relative humidities from 90 to 100 per cent. During the first six to seven days of storage, the germination was as good as that of fresh pollen.

A method of packing sugarcane pollen for long-distance shipments by air is described. Pollen of a cold-resistant wild sugarcane was shipped from Washington, D. C., to Palmira, Colombia, South America, where it was used in crosses with tropical commercial sugarcanes. An abundance of fertile hybrid seed was obtained.

Sugarcane pollen stored for four to seven days retained its ability to fertilize.

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LITERATURE CITED

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A QUANTITATIVE MORPHOLOGICAL ANALYSIS OF LARGE AND SMALL LEAVES OF ALFALFA, WITH SPECIAL REFERENCE TO INTERNAL SURFACE ¹

Franklin M. Turrell

HILL AND Thomas (1933) observed that, when leaves of alfalfa (*Medicago sativa* L.) were subjected to sulphur dioxide gas, large leaves were more readily injured than small leaves, and that neither type was injured near the veins. Zimmerman and Crocker (1934) reported similar responses in leaves of alfalfa and of several other plants.

Large leaves of *Catalpa speciosa* Warder have been shown to have greater internal-external surface ratios than smaller leaves of catalpa grown under the same conditions, and palisade tissue of leaves has been shown to expose more surface per unit volume than sponge tissue (Turrell, 1936). The extent of an absorbing area is known to be one of the factors limiting the rate of absorption of gases (Adeney and Becker, 1919, 1921).² It seemed possible, therefore, that the greater degree of injury to the large leaves of alfalfa might be related to larger internally exposed surfaces in these leaves, and that smaller internally exposed surfaces in the region of the veins of the larger leaves, as well as in the intervacular interval of smaller leaves, might be related to immunity.

Brown and Escombe (1900), in studies on the static diffusion of gases in relation to the assimilation of carbon and translocation in plants, found that the dimensions and distribution of small apertures influenced the rate of diffusion. Differences in the dimensions and distribution of stomata in large and small leaves of alfalfa might be expected, with resulting differential diffusion of sulphur dioxide; this might account for the different degree of injury in the two types of leaves. Under conditions causing closure of the stomata, injury to the alfalfa leaf might be effected either by gas penetrations of the cuticle and epidermis or by the quantity of gas trapped in the intercellular spaces.

As more sulphur seems to be excreted by roots of fumigated plants than by those of nonfumigated

plants (Thomas and Hill, 1937), it is evident that some form of sulphur ion is transported to the veins. The distance the sulphur radical is transported through the chlorenchyma may be related to susceptibility to sulphur dioxide injury. A thorough discussion of the relation of the intervacular interval to translocation in the leaf has been presented by Wylie (1939).

The present study on the quantitative morphology of alfalfa leaves is an outgrowth of interest in the responses of alfalfa leaves to sulphur dioxide gas.

The author became interested in this problem through Dr. George R. Hill, Jr., of the American Smelting and Refining Company, and wishes to express grateful appreciation to him for his unfailing kindness and for supplying the leaf samples and the outline drawings of the leaflets and stems used in these studies. Appreciation is expressed, also, to the American Smelting and Refining Company for supplying the grant which made the initiation of this work possible; to Professor R. B. Wylie for his helpful suggestions with respect to leaf structure; to Professors R. A. Fisher, G. W. Snedecor and Gertrude Cox, and to Dr. A. E. Brandt for advice with respect to statistics; and to Professor E. T. Bartholomew, Head of the Division of Plant Physiology of the University of California Citrus Experiment Station, where a portion of this work was completed.

MATERIALS AND METHODS.—Alfalfa plants for these studies were obtained from plots used for sulphur dioxide experiments by the American Smelting and Refining Company at Salt Lake City, Utah. Each plot contained twenty plants (Utah, common variety alfalfa). Four control plots were chosen because of the uniform growth of plants in these plots. Four plants similar in size and growth were picked—one from each plot; and from each plant, one of its several stems was selected. Diagrammatic drawings of these four stems, in figure 1, show the position of the leaves selected. The plants were near maturity and were from the second crop.

The first leaves to be developed on the main stem are classed as early primary leaves; those developed on the main stem later, that is, near the top, are classed as late primary. When the axillary bud from a primary leaf starts to grow, it puts out a shoot having leaves classed as secondary. On the basis of an

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² The equation for the rate of solution of gases in liquids is

$$\frac{dw}{dt} = SAp - \frac{fAw}{V},$$

where S = initial rate of solution per unit area, f = coefficient of escape, A = surface area, V = liquid volume, p = gas pressure, w = weight of gas involved, and t = time.

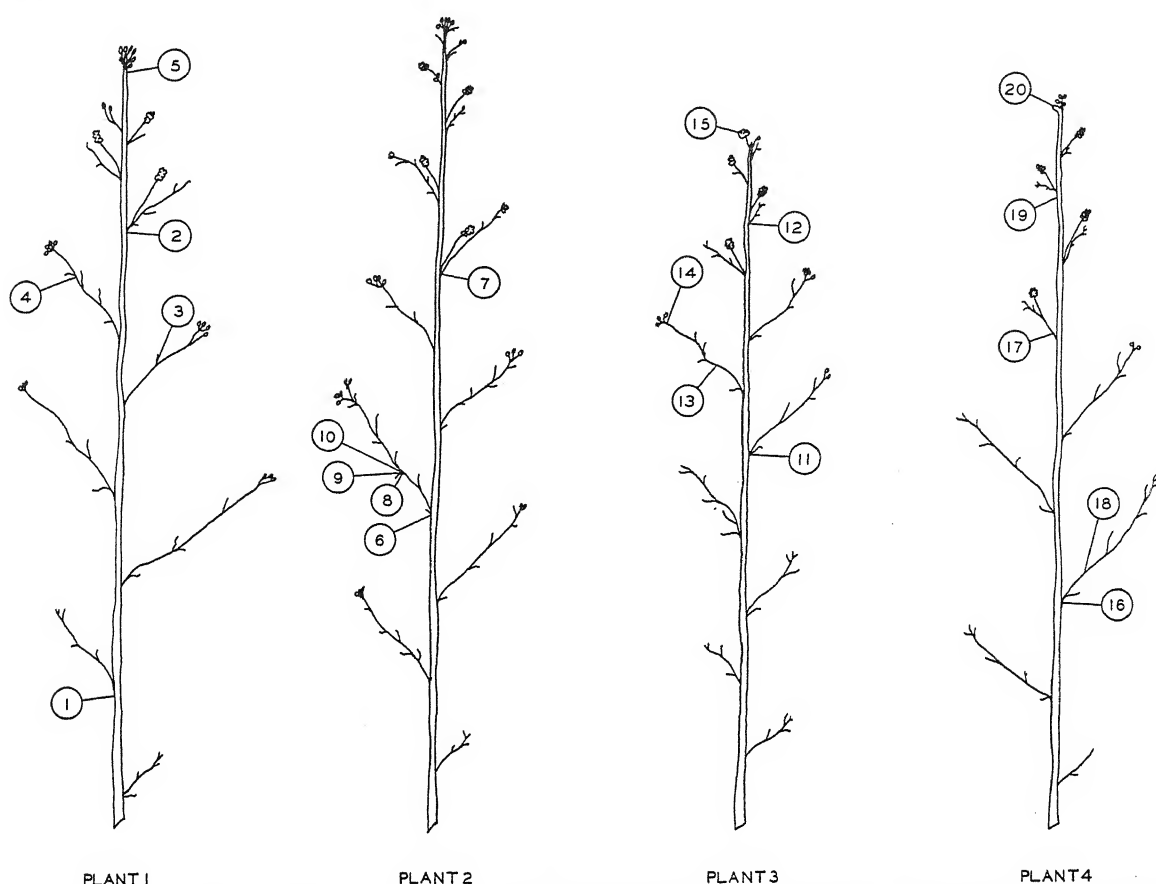


Fig. 1. Diagrammatic drawings, to scale in inches, of four alfalfa stems from four plants, showing the positions from which leaves were picked. Reduced. ($\times 7.3$.)

extension of such a system, tertiary and quaternary leaves, also, are developed. Leaves of these five

TABLE 1. *Classification and size of selected alfalfa leaves.*

Stem no.	Leaf no.	Class	Size
1 (plant 1).....	1	Early primary	Large
	2	Late primary	Large
	3	Secondary	Small
	4	Tertiary	Small
	5	Quaternary	Small
2 (plant 2).....	6	Early primary	Large
	7	Late primary	Large
	8	Secondary	Large
	9	Tertiary	Small
	10	Quaternary	Small
3 (plant 3).....	11	Early primary	Large
	12	Late primary	Large
	13	Secondary	Large
	14	Tertiary	Small
	15	Quaternary	Small
4 (plant 4).....	16	Early primary	Large
	17	Late primary	Large
	18	Secondary	Small
	19	Tertiary	Small
	20	Quaternary	Small

classes were selected from each stem as shown in table 1. The leaves were rapidly outlined, and one leaflet of each triad composing the leaf was selected. Leaves one square centimeter in area were arbitrarily classed as large leaves; those smaller in area were classed as small leaves.

A block of tissue 0.25 cm. square was cut from the center of the blade, as shown in figure 2, and killed in formalin-acetic acid-ethanol mixture. Twenty-four hours later the leaves were transferred to n-butyl alcohol solutions (Zirkle, 1930), dehydrated, and finally imbedded in paraffin. Transverse and tangential sections were cut 12 microns thick. Subsequently, the sections were stained in Delafield's hematoxylin and safranin, and mounted permanently.

All microscopical measurements and camera lucida drawings (used for chartometer and planimeter measurements) were made from these prepared slides. Measurements and drawings were made of tissue chosen at random on the slide and are representative of small regions in the central or near-central portions of the blade only (fig. 2).

Measurement of leaf area.—The penciled tracings of the selected leaflets were measured with a planimeter. Duplicate readings were made which checked within 0.02 square inches.

Measurement of internal surface.—The calculation of the ratio R (the internal surface of the leaf bordering intercellular space, measured in square microns, divided by a unit of external surface) was made with a slight modification of the method outlined by the author in an earlier paper (Turrell, 1936).

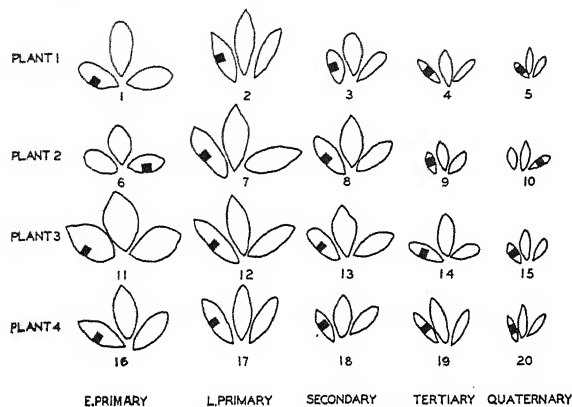


Fig. 2. Outline drawings of the leaflets of the twenty alfalfa leaves selected for these studies, showing the relative size of the leaflets, the leaflets sampled, and the positions from which the samples were taken.

Irregularity in the length of the palisade layers made it undesirable to measure each layer separately; the tissue was, therefore, handled as a unit. To distinguish the area of the internal surface of the palisade tissue thus determined, from determinations made by the original method (Turrell, 1936), the notation $l' p'$ was adopted. The presence of a layer of palisade-like tissue lying along the lower epidermis required the addition of measurements $l_1' p_1'$ which were made from camera lucida drawings in the usual manner.

Estimated internal surface in the vein region.—The vertical diameters of the major veins were measured from the transverse leaf sections. An average of these values was substituted for d_{va} in the formula given below. Similarly, the vertical diameters of the minor veins were obtained, averaged, and substituted for d_{vi} . By the use of these values and d_s (the thickness of the sponge parenchyma tissues), l' , l_1' , and R (the internal-external surface ratio for the leaf tissue within the intervacular interval), R_{va} (the internal-external surface ratio in the region of the major veins) and R_{vi} (the internal-external surface ratio of the tissue in the region of the minor veins) were calculated from the formulas:

$$(1) R_{va} = R - R \frac{d_{va}}{l' + l_1' + d_s}$$

$$(2) R_{vi} = R - R \frac{d_{vi}}{l' + l_1' + d_s}$$

Size of vein islets.—Measurements of vein islets were made on tangential sections. A microscope fitted with a 30 \times objective and 12 \times ocular was used in determining the diameters of the intervacular interval (the distance between minor veins). The

measurements were made inclusive of the border parenchyma and extended from vein edge to vein edge. Both length and width were included. Accuracy of approximately 2.27 microns could be obtained, but since the vein islets were of various shapes, ranging from triangles to irregular trapezoids, the significance of accuracy of the optical system may be minimized. The most consistent conformation was the rectangle. All measurements were made from the midpoint on any side to the midpoint on the opposite side.

Size of stomatal pores.—Pore measurements were made on camera lucida drawings of tangential sections of leaves. The microscope was fitted with a 91 \times oil-immersion objective, and a 10 \times ocular. Estimated accuracy was 0.1 micron.

Stomatal density.—Stomatal density was determined from tangential leaf sections. A microscope equipped with a high, dry, 62 \times objective and a 12 \times ocular was used. The diameter of the field was measured by the use of a stage micrometer. One hundred and fifty-five counts were made in the upper epidermis.

Size of epidermal cells.—Epidermal cell size was estimated from camera lucida drawings of the upper epidermal portions included in tangential sections. The drawings were made with a 10 \times ocular and 47.5 \times objective. Areas of ten cells were measured with a planimeter. The small subsidiary cells adjacent to stomata were never included. Samples of the remaining cells were taken at random for measurement.

Tissue thickness.—Tissue thickness was measured with a 40 \times or 90 \times objective (according to the size of the tissue) in combination with a 10 \times ocular and an ocular micrometer. Good accuracy was obtained for the 10 to 20 samples taken per leaf. Measurements were made from transverse sections.

RESULTS.—Reference to table 2 shows that the mean leaf area of the group of leaves classed as large was greater than that of the group classed as small, and that the mean value of the internal-external surface ratio in the intervacular interval was greater for the leaves of large leaf area than for those of small leaf area.

The mean areas of the leaves grouped according to origin, in square centimeters, are shown in table 3.

Estimations of the internal-external surface ratios in the regions of both major and minor veins are given in table 2. It should be noted (1) that the internal-external surface ratio in the region of the major vein of both large and small leaves is lower than the ratio in the intervacular interval or in the region of the minor veins of these leaves, and (2) that the internal-external surface ratio in the region of the major vein of the large leaves is lower than the internal-external surface ratio in the intervacular interval of the small leaves.

When the leaves were grouped according to origin, the early and late primary leaves had not only greater mean leaf area than the other leaf groups, but larger internal-external surface ratios in the

TABLE 2. *The internal-external surface ratios in the intervacular interval (R), in the region of the major vein (R_{va}), and in the region of the minor vein (R_{vi}) in large and small leaves of alfalfa, compared with leaf areas.*

Large leaves					Small leaves				
Leaf no.	R	R_{va}	R_{vi}	Leaf area, in sq. cm.	Leaf no.	R	R_{va}	R_{vi}	Leaf area, in sq. cm.
1	11.93	6.63	9.83	1.68	3	10.30	4.01	8.91	0.71
2	12.03	5.74	9.68	1.55	4	12.00	5.25	9.79	0.52
6	16.44	7.38	13.58	1.29	5	9.89	2.76	8.10	0.32
7	16.82	6.30	14.76	2.52	9	8.54	1.97	7.40	0.39
8	12.34	2.36	9.40	1.61	10	8.54	2.80	6.88	0.32
11	12.92	1.80	10.22	3.16	14	9.44	2.33	7.73	0.97
12	12.88	3.41	11.10	2.45	15	9.98	1.79	8.21	0.39
13	9.40	4.00	7.94	1.42	18	11.70	5.48	9.75	0.84
16	17.67	9.70	15.36	1.80	19	11.30	5.83	9.45	0.71
17	15.48	8.04	13.14	1.68	20	11.10	3.56	9.05	0.39
Mean	13.80	5.54	11.50	1.92	Mean	10.28	3.58	8.52	0.56

intervascular interval (R), in the major vein region (R_{va}), and in the minor vein region (R_{vi}), as shown in table 3. Secondary, tertiary, and quaternary leaf groups of successively smaller areas had successively smaller internal-external surface ratios in the intervacular interval (R), in the minor-vein region (R_{vi}), and in the major-vein region (R_{va}). The internal-external surface ratio in each leaf group was

TABLE 3. *Mean internal-external surface ratios in the intervacular area (R), in the region of the major vein (R_{va}), and in the region of the minor vein (R_{vi}) in the different alfalfa leaf groups, in comparison with mean leaf areas.*

Leaf groups	Mean internal-external surface ratios			Mean leaf area in sq. cm.
	R	R_{va}	R_{vi}	
Early primary	14.75	6.38	12.29	1.98
Late primary	14.30	5.87	12.17	2.05
Secondary	10.95	3.96	8.98	1.15
Tertiary	10.32	3.85	8.59	0.65
Quaternary	9.88	2.73	8.06	0.36

TABLE 4. *Thickness of upper and lower epidermis and of palisade and sponge tissues of large and small leaves of alfalfa, in comparison with total leaf thickness, in microns.*

Large leaves						Small leaves					
Leaf no.	Upper epidermis ^a	Palisade tissue	Sponge tissue	Lower epidermis ^a	Total leaf thickness ^a	Leaf no.	Upper epidermis ^a	Palisade tissue	Sponge tissue	Lower epidermis ^a	Total leaf thickness ^a
1	15.2	96.0	71.9	13.3	196	3	12.7	63.6	55.6	14.9	147
2	11.6	81.3	72.5	11.1	177	4	12.8	70.5	65.0	13.4	162
6	16.7	129.0	93.9	16.4	256	5	11.4	63.1	64.4	12.7	152
7	15.1	122.0	88.2	13.5	239	9	13.5	57.6	51.0	14.0	136
8	14.2	87.2	66.9	13.4	182	10	15.0	49.1	46.4	11.7	122
11	15.2	99.9	60.0	16.8	192	14	11.5	58.2	44.4	13.2	127
12	14.4	101.2	72.5	14.6	203	15	8.7	49.5	50.6	11.4	120
13	14.3	72.7	47.4	12.3	147	18	12.7	69.5	66.9	11.4	161
16	13.4	124.3	94.4	15.3	247	19	10.4	83.8	74.4	10.4	179
17	12.9	106.1	72.5	11.7	203	20	10.5	56.8	68.8	11.5	148
Mean	14.3	102.0	74.0	13.8	204.2	Mean	11.9	62.2	58.8	12.5	145.4

^a Values include cuticle thickness.

greatest in the intervacular interval (R), intermediate in the region of the minor veins (R_{vi}), and smallest in the region of the major veins (R_{va}).

Large leaves are thicker and have thicker upper and lower epidermis than small leaves (table 4 and fig. 3-7), but the percentage of total leaf thickness occupied by upper and lower epidermis is larger in small leaves (table 5).

Of the leaves classified according to origin, the early primary leaves were thickest, averaging 222.7 microns. Late primary leaves averaged 205.5 microns in total thickness; secondary leaves, 159.2 microns; tertiary leaves, 151.0 microns; and quaternary leaves, 135.5 microns. The average thickness of the upper epidermis of these leaf groups was 15.12, 13.50, 13.47, 12.05, and 11.40 microns respectively; that of the lower epidermis of the same leaf groups was 15.45, 12.72, 13.00, 12.75, and 11.42 microns, respectively.

Thickness of both palisade and sponge tissues was greater in large leaves than in small (table 4 and fig. 3-7).

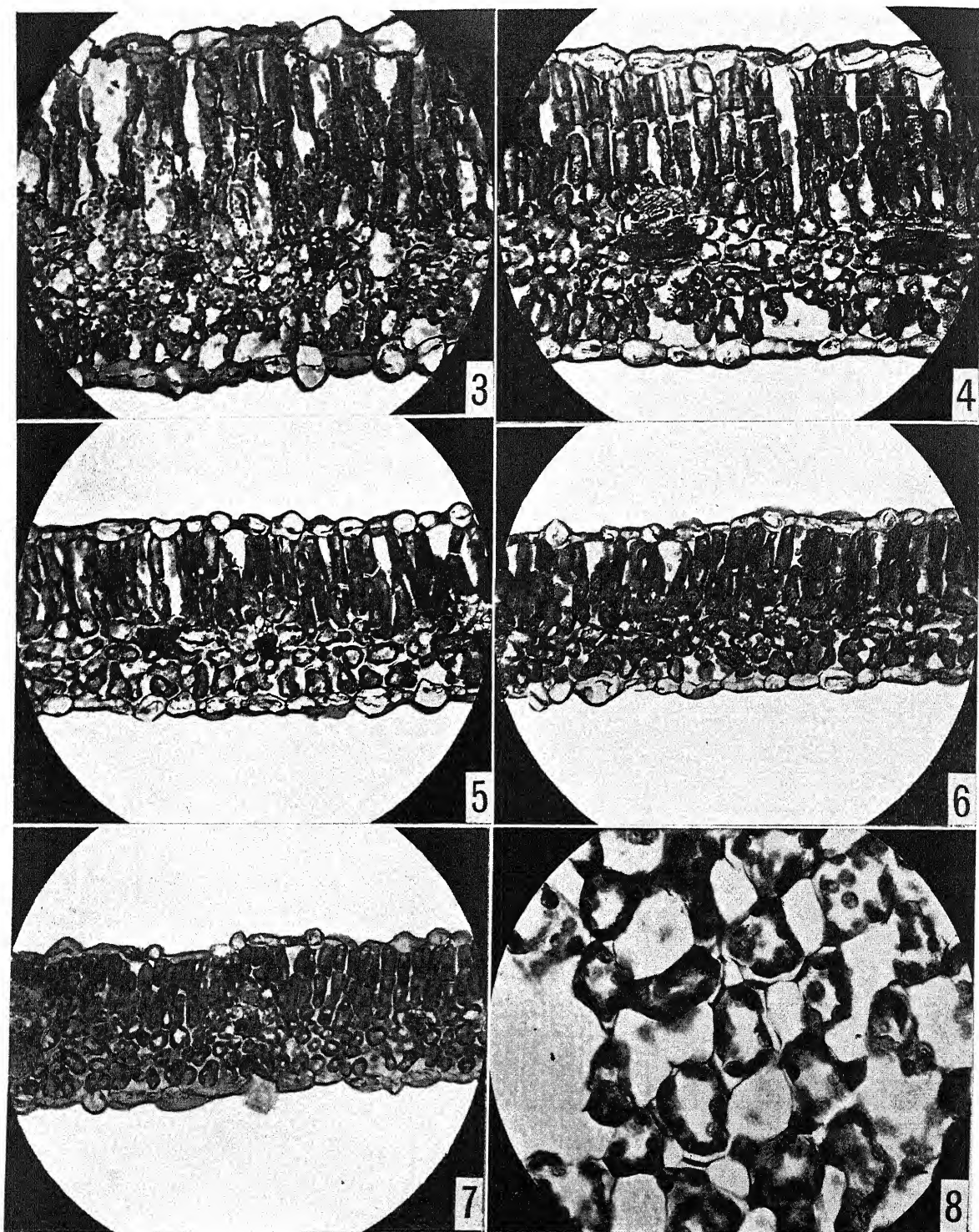


Fig. 3-8.—Fig. 3-7. Photomicrographs of transverse leaf sections, showing relative thickness of epidermal, palisade, and sponge tissues and intercellular space in the five types of leaves.—Fig. 3. Early primary leaf (no. 6). $\times 204$.—Fig. 4. Late primary leaf (no. 7). $\times 204$.—Fig. 5. Secondary leaf (no. 13). $\times 204$.—Fig. 6. Tertiary leaf (no. 14). $\times 204$.—Fig. 7. Quaternary leaf (no. 15). $\times 204$.—Fig. 8. Photomicrograph of tangential leaf section, showing type of leaf tissue measured. Sponge layer of large leaf (no. 2). $\times 627$.

TABLE 5. *Relative thickness of alfalfa leaf tissues, in percentage of total leaf thickness.*

Large leaves					Small leaves				
Leaf no.	Upper epidermal tissue	Palisade tissue	Sponge tissue	Lower epidermal tissue	Leaf no.	Upper epidermal tissue	Palisade tissue	Sponge tissue	Lower epidermal tissue
1	7.76	48.9	36.6	6.79	3	8.64	43.3	37.8	10.10
2	6.56	45.8	40.9	6.27	4	7.90	43.2	40.1	8.27
6	6.55	50.4	36.6	6.41	5	7.51	41.5	42.4	8.36
7	6.32	51.1	36.9	5.65	9	9.04	42.4	37.5	10.30
8	7.81	47.8	36.7	7.36	10	12.30	40.2	38.0	9.59
11	7.92	50.2	31.2	8.75	14	9.06	45.8	34.9	10.40
12	7.09	49.9	36.7	7.19	15	7.25	41.2	42.2	9.51
13	9.73	49.4	32.2	8.36	18	7.89	43.1	41.5	7.08
16	5.42	50.4	38.2	6.19	19	5.81	46.8	41.6	5.81
17	6.35	52.3	35.7	5.76	20	7.09	38.4	46.5	7.76
Mean	7.15	49.6	36.2	6.87	Mean	8.25	42.6	40.3	8.72

The average thickness of the palisade tissue in the different leaf groups, in microns, was as follows: early primary, 112.30; late primary, 102.15; secondary, 73.25; tertiary, 67.52; and quaternary, 54.62. Thickness of sponge tissue in these groups averaged 80.05, 76.42, 59.20, 58.70, and 57.55 microns, respectively. Mesophyll thickness (that is, palisade thickness plus sponge thickness) in these groups of leaves averaged 192.25, 179.25, 132.25, 126.25, and 112.50 microns, respectively. The relative thicknesses of these tissues are shown in figures 3-7.

The percentage of leaf thickness occupied by palisade tissue was larger in large leaves than in small, while the percentage of sponge tissue was larger in small leaves (table 5). As may be readily calculated from table 5, the ratio of percentage of palisade tissue to sponge tissue was larger in the large leaves than in small.

Values recorded in table 6 show the area of internal surface, in square microns, exposed to intercellular space per cubic micron of leaf tissue (volume of cells plus volume of intercellular space). In the large leaves palisade tissue is only 0.96 times as efficient as sponge tissue in exposing cellular surface, while in the small leaves the palisade tissue is 0.97

times as efficient as sponge tissue. Comparison of the mean values in table 6 shows that in both palisade and sponge tissues small leaves have a greater exposure of internal surface per unit volume than large leaves.

Early primary, late primary, secondary, tertiary, and quaternary leaves had an internal palisade surface of 0.156, 0.153, 0.161, 0.162, and 0.169 square microns per cubic micron of palisade tissue, respectively. These same groups of leaves had an internal sponge surface of 0.149, 0.167, 0.171, 0.169, and 0.172 square microns per cubic micron of sponge tissue, respectively.

The mean stomatal density in small leaves was greater than in large leaves (table 7). Early primary, late primary, secondary, tertiary, and quaternary leaves had 215.4, 217.3, 214.0, 266.5, and 312.4 stomata per square millimeter, respectively. Stomata are shown in figures 9, 10, 15, and 16.

The average size of stomatal pores in the large leaves was 8.1 by 2.1 microns, and in the small leaves, 6.7 by 1.6 microns. The average interstomatal distances were 68 microns in the large leaves and 60 microns in the small leaves. (Compare fig. 9 and 10, 15 and 16.)

TABLE 6. *Internal surface (square microns) per unit volume (cubic microns) for palisade and sponge tissues of large and small leaves of alfalfa.*

Large leaves			Small leaves		
Leaf no.	Palisade tissue	Sponge tissue	Leaf no.	Palisade tissue	Sponge tissue
1	0.149	0.133	3	0.184	0.161
2	0.132	0.183	4	0.179	0.178
6	0.160	0.130	5	0.138	0.172
7	0.154	0.168	9	0.144	0.172
8	0.145	0.181	10	0.178	0.180
11	0.166	0.154	14	0.195	0.168
12	0.151	0.144	15	0.187	0.155
13	0.156	0.157	18	0.158	0.186
16	0.148	0.179	19	0.129	0.158
17	0.173	0.173	20	0.171	0.181
Mean	0.153	0.160	Mean	0.166	0.171

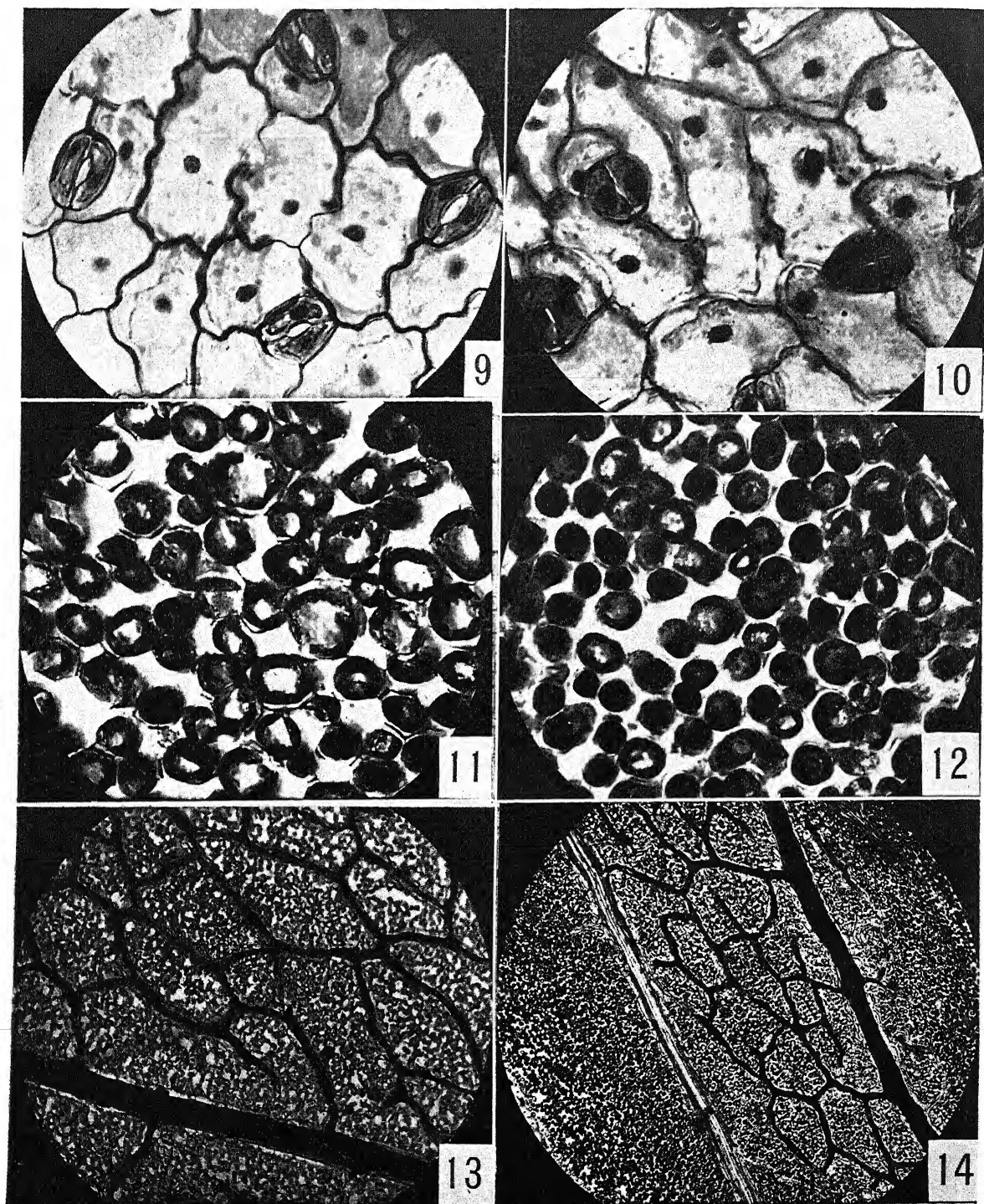


Fig. 9-14. Photomicrographs of tangential leaf sections, showing types of leaf tissue measured.—Fig. 9. Upper epidermis of large leaf (no. 2), showing stomata. $\times 579$.—Fig. 10. Upper epidermis of small leaf (no. 15), showing stomata. $\times 579$.—Fig. 11. Palisade layer of large leaf (no. 11), showing intercellular space. $\times 579$.—Fig. 12. Palisade layer of small leaf (no. 15), showing intercellular space. $\times 579$.—Fig. 13. Sponge layer of large leaf (no. 2), showing the vein islets. $\times 191$.—Fig. 14. Sponge layer of small leaf (no. 15), showing the vein islets. $\times 191$.

TABLE 7. *Density and pore size of stomata in large and small leaves of alfalfa.*

Large leaves				Small leaves			
Leaf no.	Number of stomata per sq. mm.	Pore size		Leaf no.	Number of stomata per sq. mm.	Pore size	
		L ^a	W ^a			L ^a	W ^a
1	221.2	7.4	1.9	3	219.0	6.4	1.7
2	267.6	7.0	1.9	4	318.5	5.5	1.5
6	215.7	7.3	1.5	5	276.5	6.1	1.7
7	199.0	7.7	2.2	9	227.8	5.5	1.4
8	230.0	10.2	2.2	10	265.4	7.0	1.2
11	223.4	8.2	2.1	14	265.4	5.9	1.4
12	170.3	9.2	2.4	15	318.5	5.1	1.0
13	201.3	7.2	1.8	18	205.7	8.5	2.3
16	201.3	9.9	2.9	19	254.4	8.5	2.3
17	232.3	7.2	2.3	20	389.3	8.3	1.5
Mean	216.2	8.1	2.1	Mean	274.1	6.7	1.6

^a Length and width in microns.

The mean diameters of midrib, lateral veins, and minor veins respectively, are greater in the large leaves than in the small leaves (table 8). Diameters of midribs of early primary, late primary, secondary, tertiary, and quaternary leaves were 145.7, 151.0, 115.9, 99.5, and 101.2 microns, respectively. Diameters of major veins of these groups of leaves were 108.5, 106.5, 84.7, 78.5, and 81.2 microns, while those of minor veins were 32.7, 26.5, 24.0, 21.2, and 20.7 microns, respectively.

Distances between major lateral veins in large leaves averaged 933.1 microns, and in small leaves, 611.0 microns (table 8).

The mean length and mean width of the unit of chlorenchyma (mesophyll) surrounded by minor veins (vein-islet or intervacular interval) was larger in the large leaves than in the small leaves (table 8). Compare vein islets illustrated in figures 13 and 14.

Dimensions (mean length by mean width) of vein islets for the leaf groups were as follows: early pri-

mary, 253×123 microns; late primary, 220×125 microns; secondary, 230×105 microns; tertiary, 168×92 microns; and quaternary, 167×76 microns.

Leaf tissue of large leaves, exclusive of veins, contained a greater mean volume of intercellular space than that of small leaves (table 9). The mean percentage volume of intercellular space was also larger in large leaves than in small (table 9). Calculations based on this table show that the mean volumes of intercellular space in samples from leaves of the different leaf groups, in cubic microns, were as follows: early primary, 240,305; late primary, 197,938; secondary, 153,862; tertiary, 120,937; and quaternary, 98,034. The percentage volumes of intercellular space for these same groups of leaves were 29.4, 26.5, 26.9, 23.2, and 19.9, respectively. Intercellular space in cross sections of leaves is shown in figures 3-7, and in tangential sections, in figures 8, 11-14, 17-20.

DISCUSSION.—*Errors in methods.*—Errors involved in the measurement of internal surface were

TABLE 8. *Vein diameters, distances between major lateral veins, and dimensions of vein islets in large and small leaves of alfalfa, expressed in microns.*

Large leaves							Small leaves						
Leaf no.	Midrib diam-eter	Major-vein diam-eter	Minor-vein diam-eter	Distance between major veins	Vein-islet length	Vein-islet width	Leaf no.	Midrib diam-eter	Major-vein diam-eter	Minor-vein diam-eter	Distance between major veins	Vein-islet length	Vein-islet width
1	126	75	29	596	263	130	3	120	73	17	755	248	100
2	144	81	30	884	213	132	4	117	76	25	390	149	85
6	165	123	40	1,272	150	97	5	109	92	23	— ^a	180	67
7	202	131	25	807	212	109	9	73	84	15	281	160	109
8	164	125	37	486	184	99	10	86	65	19	— ^a	175	78
11	174	137	33	1,069	257	142	14	97	78	19	501	192	81
12	155	128	24	974	228	143	15	109	82	18	— ^a	155	74
13	86	69	19	939	240	109	18	94	72	23	1,069	247	113
16	118	99	29	1,415	342	121	19	111	76	26	673	171	91
17	103	86	27	789	227	114	20	101	86	23	— ^a	156	84
Mean	143.7	105.4	29.3	933.1	231.6	119.6	Mean	101.7	78.4	20.8	611.0	183.3	88.2

^a Dashes indicate data not available.

given by Turrell (1936). They include the maximum error in drawing with the camera lucida, measurement of drawings with chartometer and planimeter, and measurement of the tissues directly with an ocular micrometer. The earlier work was based on single

internal surface and since no data on internal surface were available prior to this study, bias in selection of leaves for internal-surface measurements would be impossible.

From each of the five leaves of plant no. 2, five samples of tissue were chosen at random for every item in the formula used for measuring the internal leaf surface, and were drawn with the camera lucida. These drawings were measured first by the author and then by another investigator. It was thought that a better estimate of error for the method in the hands of various investigators would be obtained by means of measurements made by a second investigator than by means of duplicate measurements made by the author. Duplicate measurements of the internal-external surface ratio in the intervascular interval (R), determined by the two investigators, are given in table 10.

The variance between means of R for the leaves of plant no. 2 is significantly greater than the variance of R within leaves.

A more critical test of the precision of the method of measuring R would be to distinguish between samples chosen at random in the same area of a given leaf rather than between samples from different leaves of the same plant. Snedecor (1934) has outlined a method by which this may be accomplished. While application of his method does not give the error of method of measuring R , it gives an estimate of one of the largest errors in the method, namely, the application of instruments to measuring drawings.

The "within-leaves" comparison contains three of the four variances contained in the "between means-of-leaves" comparison: (1) the variance due to structural variation within the leaf, (2) the variance due to making drawings, and (3) the variance due to measuring the drawing with instruments. Since duplicate drawings were not made of the same samples of leaf tissue, it is not possible to separate structural variation of the tissue from variations in the precision of the drawings; but the degree to which these two ($V.B.$) contributed to the within-leaves variance, as compared with that due to the measurement of drawings ($V.M.$), can be estimated.

$$\begin{aligned} 2 V.B. + 0.36 &= 2.96 \\ V.B. &= 1.30 \\ \frac{V.B.}{V.M.} &= \frac{1.30}{0.36} = 3.61 \end{aligned}$$

This shows that the structural variation along with the small variations in drawing is 3.61 times as large as the variance in the measurements made by the two investigators. This lends confidence to the results obtained by the method of measuring R .

Leaf-growth correlations.—A brief study of the method of growth of alfalfa showed that the number of leaves produced per plant and per node increased with the age of the plant. For instance, a stem with six nodes carried as many primary leaves, while a stem with eight or nine nodes had secondary leaves arising from several of the older nodes having pri-

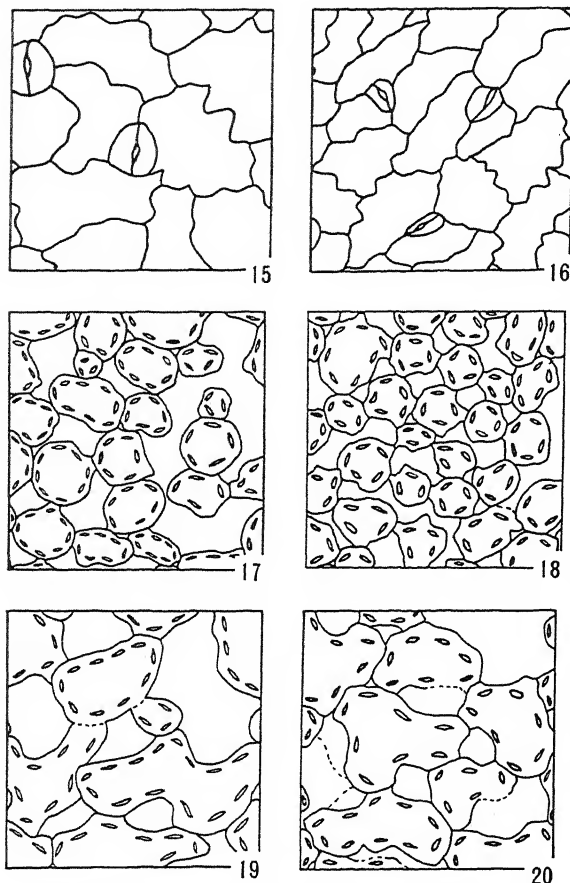


Fig. 15-20. Camera-lucida drawings of tissues from a large leaf (early primary, no. 6) and a small leaf (quaternary, no. 10) of alfalfa, drawn to the same scale and showing the larger sizes of guard, epidermal, palisade, and sponge cells and the larger intercellular spaces in the large leaf.—Fig. 15. Surface view of upper epidermis of large leaf.—Fig. 16. Surface view of upper epidermis of small leaf.—Fig. 17. Tangential section in palisade tissue of large leaf.—Fig. 18. Tangential section in palisade tissue of small leaf.—Fig. 19. Tangential section in sponge tissue of large leaf.—Fig. 20. Tangential section in sponge tissue of small leaf.

leaves which were selected to give as near maximum values as possible and yet be comparable between species. The present work on a single species involves the investigation of twenty leaves of various sizes from four plants and random measurements within the central areas of these leaves.

A statistical estimation of the magnitude of some of the sources of error in the measurement of internal surface and of their significance can be made, for instance, on plant no. 2. Since the leaves in this study were selected on the basis of origin rather than of

TABLE 9. *Comparative volumes of intercellular space in tissue of large and small leaves of alfalfa. Samples measured were 3,600 square microns of leaf surface by leaf depth.*

Large leaves			Small leaves		
Leaf no.	Volume of intercellular space per sample, in cubic microns	Percentage volume of intercellular space	Leaf no.	Volume of intercellular space per sample, in cubic microns	Percentage volume of intercellular space
1	227,285	32.2	3	157,167	29.7
2	153,424	24.1	4	113,617	22.9
6	311,549	33.8	5	116,191	21.2
7	267,644	31.1	9	130,250	26.6
8	166,936	25.5	10	85,637	19.5
11	128,680	18.6	14	93,453	20.4
12	149,284	20.4	15	76,595	17.7
13	135,761	25.7	18	155,582	26.8
16	293,706	33.0	19	146,426	22.7
17	221,401	30.3	20	113,712	21.3
Mean	205,567	27.5	Mean	118,863	22.9

mary leaves. A plant nearing maturity and having twelve or more nodes bore, at the oldest nodes, tertiary leaves as well as primary and secondary leaves. And a plant having fifteen or more nodes bore many quaternary leaves at the oldest nodes, in addition to the primary, secondary, and tertiary leaves.

First, second, third, or fourth leaves, arising at a given node, or from an axis at a given node, are readily distinguished by the length of their petioles and the size of their leaflets, the latest arrivals having, in general, the shortest petiole and smallest leaflet areas. Thus early primary, late primary, secondary, tertiary, and quaternary leaves at any one node are of decreasing ages. This is reflected clearly in the leaf structure.

Mounts (1932) showed that during leaf maturation the epidermal cells expanded more rapidly than the mesophyll cells and caused the development of intercellular spaces. On this basis, all primary leaves and some secondary leaves may have characteristics of mature leaves (large leaves); while some second-

ary, all tertiary, and all quaternary leaves have characteristics of immature leaves (small leaves) when the plant is near maturity. This is shown by comparison of values in table 11 for large and small leaves. (Though small leaves, having been arrested in growth, have characteristics of immature leaves, they may be quite as mature as the large leaves.) For instance, the ratio of the mean area of upper epidermal cells (ten cells per leaf) of the large leaves (1,446 square microns) to that of the small leaves (1,133 square microns) is 1.28, which shows that the epidermal cells of large leaves expanded 1.28 times in area. A comparison of equal areas in tangential sections of sponge tissue indicates that the area of sponge cells per unit area is only 0.945 as much in large leaves (2,563 square microns) as in small (2,714 square microns). The cellular area per unit area in the epidermis increased, while that in the sponge tissue decreased; thus intercellular spaces were produced. This is illustrated in figures 15-20. These relationships may be more clearly un-

TABLE 10. *Duplicate determinations^a of the internal-external surface ratios (R) for five samples from each of five different alfalfa leaves selected from a single stem.*

Sample no.	Investigator	Leaf no. 6	Leaf no. 7	Leaf no. 8	Leaf no. 9	Leaf no. 10
1	{a	16.09	15.16	11.61	8.33	8.29
	{b	16.06	15.01	11.92	7.47	8.33
2	{a	15.70	15.59	13.26	8.86	10.03
	{b	15.25	18.18	12.58	7.80	9.27
3	{a	19.92	15.63	13.06	8.79	8.47
	{b	18.92	14.16	12.94	8.78	8.77
4	{a	15.99	19.61	12.99	9.14	8.96
	{b	16.43	18.11	12.61	9.57	9.38
5	{a	17.33	15.92	12.28	8.52	7.97
	{b	18.33	16.84	12.44	8.42	7.56
Average	{a	17.006	16.382	12.640	8.728	8.744
	{b	16.998	16.460	12.498	8.408	8.642

^a Determinations were made by two investigators, a and b, independently.

TABLE 11. *Length of exposed cell wall and mean cellular area in tissue samples of large and small leaves of alfalfa.*

Large leaves					Small leaves				
Leaf no.	Exposed cell wall per sample, ^a length in microns		Mean cellular area per sample, ^a in square microns		Leaf no.	Exposed cell wall per sample, ^a length in microns		Mean cellular area per sample, ^a in square microns	
	Palisade tissue	Sponge tissue	Sponge tissue	Upper epidermis		Palisade tissue	Sponge tissue	Sponge tissue	Upper epidermis
1	536	362	2,290	1,412	3	660	362	2,290	1,305
2	476	407	2,730	1,183	4	640	394	2,620	1,198
6	577	387	2,140	1,698	5	498	419	2,540	1,040
7	555	427	2,660	1,509	9	518	385	2,650	889
8	520	399	2,340	1,358	10	641	374	2,895	999
11	599	308	2,710	1,514	14	707	433	2,810	1,178
12	543	339	2,870	1,584	15	674	518	2,900	1,127
13	561	393	2,760	1,346	18	554	393	2,480	1,252
16	532	433	2,440	1,655	19	464	368	2,940	1,359
17	621	383	2,690	1,203	20	616	366	3,010	981
Mean	552.0	383.8	2,563.0	1,446.2	Mean	597.2	401.2	2,713.5	1,132.8

^a Samples of palisade and sponge tissues were measured from tangential sections and were 3,600 square microns in area. Samples of upper epidermis included ten cells each.

derstood by reference to table 12, in which it is shown that large leaves also have, on the average, fewer cells and portions of cells per unit area in adaxial palisade, abaxial palisade, and in the sponge regions than small leaves. (See also fig. 11 and 12, 17-20.)

When data in tables 11 and 12 are rearranged according to the origin of the leaf, the method of intercellular space development is even clearer. In early primary, late primary, secondary, tertiary, and quaternary leaves the total length of exposed cell walls per sample area of 3,600 square microns in tangential sections in the palisade tissue was 561.0, 548.7, 573.7, 582.2, and 607.2 microns, respectively. In the sponge tissue of the same groups of leaves, these values were 372.5, 389.0, 386.7, 395.0, and 419.2 microns, respectively. The mean cellular area per sample area of the sponge tissue for the same grouping of leaves also shows a greater concentration of

cellular material in small leaves than in large leaves.

Obaton (1921) observed that large and small leaves of the same plant genus and of the same age had approximately the same epidermal thickness, palisade thickness, and sponge thickness in each of the six woody genera studied. Large leaves of alfalfa, however, have greater thickness of epidermal, palisade, and sponge tissue than small leaves. Reference to the data in table 4 shows that this is the case. The differences in structure are clearly evident in figures 3-7.

The greater internal surface of large, mature alfalfa leaves apparently is not due to greater efficiency of palisade tissue in exposing surface, since palisade tissue is less efficient than sponge tissue in this respect (table 6).³ But the greater actual thick-

³ In woody species, Turrell (1936) found a higher efficiency of exposure in palisade tissue than in sponge tissue.

TABLE 12. *Number of entire cells and portions of cells in sample area^a in large and small leaves of alfalfa.*

Large leaves				Small leaves			
Leaf no.	Adaxial palisade tissue	Abaxial palisade tissue	Sponge tissue	Leaf no.	Adaxial palisade tissue	Abaxial palisade tissue	Sponge tissue
1	21	19	12	3	36	21	15
2	26	17	13	4	31	23	13
6	28	23	12	5	30	33	14
7	26	24	14	9	30	23	15
8	30	26	13	10	36	— ^b	13
11	34	16	9	14	40	23	14
12	30	20	11	15	42	32	20
13	29	19	16	18	26	20	10
16	25	19	13	19	21	23	13
17	29	30	14	20	30	19	13
Mean	27.8	21.3	12.7	Mean	32.2	24.1	14.0

^a Each sample consisted of a square of tissue 3,600 square microns in area.

^b No abaxial palisade tissue present in this sample.

TABLE 13. *Correlation between measurements of single tissues and aggregates of tissues of alfalfa leaves.*

Items correlated	Correlation coefficient <i>r</i>	Degrees of freedom <i>df</i>	Probability <i>p</i>
Leaf area (estimated) and upper epidermal thickness, entire sample.....	+0.440	18	<0.10
Leaf area (estimated) and lower epidermal thickness, entire sample.....	+0.541	18	<0.02
Leaf area (estimated) and palisade thickness, entire sample.....	+0.595	18	<0.01
Leaf area (estimated) and leaf thickness, entire sample.....	+0.537	18	<0.02
Leaf area (estimated) and major-vein diameter, entire sample.....	+0.579	18	<0.01
Leaf area (estimated) and minor-vein diameter, entire sample.....	+0.502	18	<0.05
Leaf area (estimated) and sponge-cell area (<i>A</i>) ^a (tangential section), entire sample..	-0.569	18	<0.01
Leaf area (estimated) and sponge exposed cell perimeters (<i>c</i>) ^a (tangential section), entire sample	-0.424	18	<0.10
Leaf area (estimated) and palisade exposed cell perimeters (<i>p</i>) ^a (tangential section), entire sample	-0.129	18	>0.10
Leaf area (estimated) and <i>R</i> , entire sample.....	+0.615	18	<0.01
Leaf area (estimated) and <i>R</i> _{ea} , entire sample	+0.272	18	>0.10
Leaf area (estimated) and <i>R</i> _{et} , entire sample	+0.586	18	<0.01
Leaf area (planimeter-measured) and <i>R</i> , entire sample	+0.502	18	<0.05
Leaf area (planimeter-measured) and early primary, late primary, secondary, tertiary, and quaternary leaf groups ^b	-0.821	18	<0.01
Mesophyll thickness and <i>R</i> , by plants, plant 1.....	+0.743	3	>0.10
Mesophyll thickness and <i>R</i> , by plants, plant 2.....	+0.989	3	<0.01
Mesophyll thickness and <i>R</i> , by plants, plant 3.....	+0.935	3	<0.02
Mesophyll thickness and <i>R</i> , by plants, plant 4.....	+0.997	3	<0.01
Mesophyll thickness and <i>R</i> , by leaf groups, early primary.....	+0.947	2	<0.10
Mesophyll thickness and <i>R</i> , by leaf groups, late primary.....	+0.916	2	<0.10
Mesophyll thickness and <i>R</i> , by leaf groups, secondary.....	+0.927	2	<0.10
Mesophyll thickness and <i>R</i> , by leaf groups, tertiary.....	+0.795	2	>0.10
Mesophyll thickness and <i>R</i> , by leaf groups, quaternary.....	+0.711	2	>0.10
Mesophyll thickness and <i>R</i> , entire sample	+0.939	18	<0.01
Mesophyll thickness and <i>R</i> _{ea} , entire sample	+0.757	18	<0.01
Mesophyll thickness and <i>R</i> _{et} , entire sample	+0.941	18	<0.01
Leaf thickness and <i>R</i> , by leaf groups, early primary.....	+0.944	2	<0.10
Leaf thickness and <i>R</i> , by leaf groups, late primary.....	+0.915	2	<0.10
Leaf thickness and <i>R</i> , by leaf groups, secondary.....	+0.927	2	<0.10
Leaf thickness and <i>R</i> , by leaf groups, tertiary.....	+0.796	2	>0.10
Leaf thickness and <i>R</i> , by leaf groups, quaternary.....	+0.708	2	>0.10
Leaf thickness and <i>R</i> , entire sample	+0.929	18	<0.01

^a "See Turrell (1936)."

^b These leaf groups were given arbitrary values as follows: primary (early and late), 1; secondary, 2; tertiary, 3; quaternary, 4 (see text, page 412).

ness of palisade and sponge tissues in large leaves accounts largely for the greater internal surface of these leaves (table 4). The effect of the thickening of the lamina in large leaves on the internal exposed surface is offset somewhat by the smaller amount of cell-wall perimeter and cellular area in samples of the tangential leaf sections (cell-wall perimeters and cellular areas have a small negative correlation with leaf area [table 13]).

The coefficient of correlation between internal-external surface ratio (*R*) and leaf thickness (table 13) is high and highly significant.⁴ The relationship is expressed by the regression equation (table 14), the standard error of estimate of which is 1.0, where *T* is leaf thickness, expressed in microns, and *R* is the internal-external surface ratio.

For leaves of alfalfa grown under the same conditions, the best linear relationship between leaf thick-

ness and leaf area is represented (fig. 21) by the regression equation (table 14), *T* representing leaf thickness in microns and *A*, leaf area in square centimeters. The standard error of estimate is 33.4 microns (table 14).

Leaf area is moderately and highly significantly correlated with *R*, as shown by the correlation coefficient (table 13). This relationship is more clearly seen in the regression line (fig. 22), the equation for which is shown in table 14, *R* representing internal-external surface ratio and *A* the area of the leaf, in square centimeters.

In table 13 the degree of correlation between estimated leaf area,⁵ mesophyll thickness, leaf thickness and various tissue thicknesses, tissue measurements, and internal-external surface ratios are also given. The probability of the chance occurrence of the correlation coefficient found is indicated as one chance

⁴ For significance of correlation coefficient see Fisher (1932).

⁵ Leaf area was not determined with planimeter for these calculations.

in one hundred ($P=0.01$), two chances in one hundred ($P=0.02$), five chances in one hundred ($P=0.05$), and ten chances in one hundred ($P=0.10$). That the correlation coefficients of estimated leaf area and other items approximate the correlation coefficients of measured leaf area and other items is shown by comparison of r for estimated leaf area and R , and r for planimeter-meas-

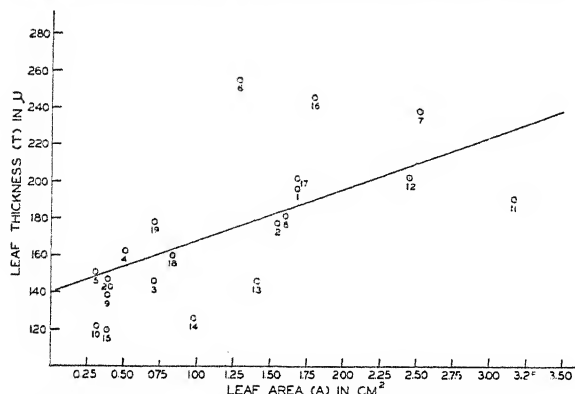


Fig. 21. The regression line of leaf thickness (T) on leaf area (A), where T is expressed in microns and A , in square centimeters.

TABLE 14. Regression equations and standard errors of estimate of certain measured items of alfalfa leaves.

Regression of	Regression equation ^a	Standard error of estimate
Leaf thickness on leaf area	$T = 140.64 + 27.72A$	33.4
R on leaf area	$R = 9.33 + 1.79A$	2.5
R_{vi} on leaf area . . .	$R_{vi} = 8.32 + 1.34A$	2.7
R on leaf thickness. .	$R = 3.97 + 0.04T$	1.0

^a T = leaf thickness in microns; A = leaf area in square centimeters.

ured leaf area and R (table 13): the former is $+0.61$ and is highly significant; the latter is $+0.50$ and is significant.

As shown in table 13, estimated leaf area is positively and moderately correlated with upper and lower epidermal thickness, palisade thickness, leaf thickness, major-vein diameter, minor-vein diameter, R , R_{va} , and R_{vi} , as indicated by the correlation coefficients, which range around $+0.5$. It is negatively correlated with the origin of the leaf in relation to the main stem. When primary leaves are arbitrarily given a value of 1; secondary leaves, 2; tertiary leaves, 3; and quaternary leaves, 4 (table 13), the correlation coefficient is -0.8 . Mesophyll thickness and R were highly and significantly correlated in three of the plants. Mesophyll thickness and R were highly but not significantly correlated in early primary, late primary, and secondary leaves; the correlation was high but not significant for tertiary and quaternary leaves. The correlation was high between mesophyll thickness and R , R_{va} , or R_{vi} , and

the correlation coefficient was highly significant. Leaf thickness and R were highly but not significantly correlated in early primary, late primary, and secondary leaves; they were moderately but not significantly correlated in tertiary and quaternary leaves. When the entire sample was considered, however, the correlation was very high and highly significant between mesophyll thickness and R , and leaf thickness and R .

The latter two correlations suggest that when a population of leaves from this variety of alfalfa is sampled, such complicated estimates as R might be made from a regression equation based on leaf area when high accuracy is not essential and based on mesophyll thickness when high accuracy is desirable.

The relation of plant maturity to SO_2 injury.—Because of the mode of growth of the alfalfa plant some relation between maturity and sulphur dioxide injury might be expected, for early in the develop-

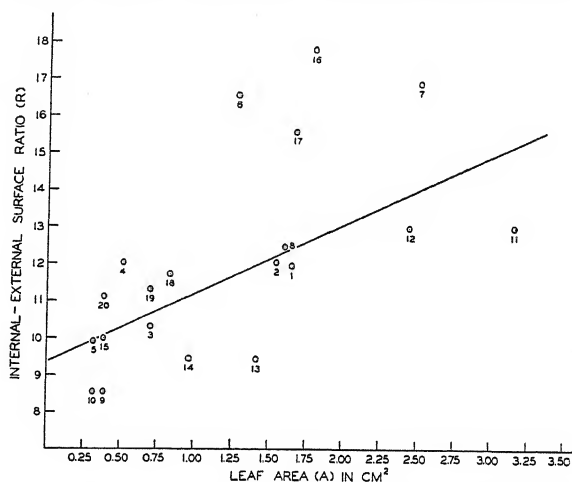


Fig. 22. The regression line of internal-external surface ratio (R) on leaf area (A), where the latter is expressed in square centimeters.

ment of the plant several primary leaves are formed; later, secondary leaves arise; increasing numbers of both are produced before tertiary leaves appear. Finally, still accompanied by production and maturation of leaf types already named, the quaternary leaves arise. Since large leaves, that is, early primary, late primary, and some secondary leaves, are readily injured, while small leaves, that is, some secondary, tertiary and quaternary are not so readily injured, it might be expected that the total leaf injury to a mature plant would always be greater than that to an immature one under the same conditions, because of the large number of large mature leaves; but the percentage injury might not necessarily always be expected to be much greater, since the quantity of small immature leaves which are not readily injured affect the percentage. Nevertheless, if the plants are selected in the correct growth stages, the percentages are also affected, as indicated by cal-

culations based on Hill's and Thomas' curves (1933) for the percentage leaf area destroyed in early growth (25 per cent to 40 per cent growing time), in medium growth (40 per cent to 60 per cent growing time), and in late growth stages which give the relative amount of leaf injury as 16.9, 23.5, and 33.6, respectively. The injury at maturity is 33.6/16.9, or 1.99 times the injury at early growth stages.

The relation of leaf structure to SO₂ injury.—The effect of maturity in sulphur dioxide injury has been based on the size of leaves, and since the size of leaves has been shown to be closely related to a number of morphological features of the leaf, these might well be examined with respect to leaf injury by sulphur dioxide gas.

Measurements show that both the upper and lower epidermis of large leaves are thicker than those of small leaves (table 4). The differences in these features may be seen in figures 3 to 7. Since large leaves show injury first, epidermal thickness and cuticle thickness, therefore, are not positively but negatively correlated with SO₂ injury, when considered barriers to SO₂ absorption.

Entrance to the intercellular space of alfalfa leaves is provided by stomata on both surfaces of the leaf and this suggests an arrangement for more rapid gaseous exchange between intercellular space and external atmosphere than in leaves having stomata only on one surface. This stomatal situation in conjunction with the very thin cell walls (fig. 8, 11 and 12) of the mesophyll, may in part explain the sensitivity of alfalfa to SO₂, as noted by Setterstrom, Zimmerman, and Crocker (1938); while the variability in the exposure of cell wall may explain why injury to the leaf occurs in spots.

In large alfalfa leaves we have shown that 27.5 per cent of the leaf sample volume was intercellular space, while in small leaves only 22.9 per cent was intercellular space. If some environmental condition caused closure of stomata during fumigation with SO₂ shortly after the gas in the intercellular space is in equilibrium with that in the external air, then large leaves would accumulate 4.6 per cent more SO₂ per unit sample volume of leaf tissue than small leaves. Since the average large leaf contains about 0.0109 cc. of intercellular space in the entire leaf, and the average small leaf contains 0.00184 cc., under the same conditions stomatal closure would cause large leaves to trap about 5.9 times more SO₂ than small leaves.

Brown and Escombe (1900) showed that the distribution of small apertures which most efficiently transmitted gases through thin membranes was where interaperture distances were about ten times the aperture diameters, in cases where there was no air movement. In large alfalfa leaves the mean interstomatal distance calculated from the stomatal density data is 68 microns and is significantly different from 60 microns for small leaves. By making further calculations according to Sayre's modification (1926) of Brown and Escombe's law, we find the diameter of a circular pore with a perimeter equal

to that of the average of elliptical stomatal pores in large leaves would be 5.9 microns, while in small leaves it would be 4.8 microns. Thus large leaves which are more readily injured by SO₂ have a more efficient distribution of stomata for transmission of gas in still air. See figures 9, 10, 15, and 16.

In moving air Brown and Escombe showed that diffusion of gas through thin membranes was proportional to the pore area. In the field where there is nearly always some air movement the significantly larger stomatal pore areas of large leaves would favor transmission of SO₂ with attending greater injury.

Thomas and Hill (1937) have noted a translocation of sulphur from fumigated leaves. Our measurements show large leaves had larger mean vein diameters in the midribs, lateral veins, and in the minor veins, respectively, than small leaves (table 8). The larger leaves thus may be better adapted for translocation than small leaves, and on this basis they would be expected to show less injury though perhaps greater absorption coefficients because of removal of products formed with SO₂. However, the protoplasm is apparently injured in the large leaves before the toxic mixture reaches the veins, so that a negative correlation exists between vein diameter and SO₂ injury.

A partial explanation of the negative correlation between vein size and sulphur dioxide absorption and injury probably lies in the greater distances which the sulphur complex must be transported before reaching the specialized conductive tracts in large leaves. Both the lengths and widths of the vein islets (mesophyll units) are larger in large leaves than in small (table 8), so that a positive correlation exists between the distance that the SO₂ must be transported to reach the veins and the degree of injury. This is probably in some way related to the internal exposed surface and will be discussed later. Difference in vein islet size is illustrated in figures 13 and 14.

As shown by analyses of variance, the large leaves were significantly greater than small leaves in the area of internal surface (R) in the intervacular interval exposed to intercellular space per unit leaf surface (7,200 square microns). And it has been noted by Hill and Thomas (1933) that the large alfalfa leaves are injured more readily and more severely than small ones. They (Thomas and Hill, 1937) have also shown that the large leaves (primary and secondary) of Utah Common Alfalfa contained approximately 2 per cent sulphur after twenty-two days of continuous fumigation with sulphur dioxide (concentration of 0.24 ppm.), while the small leaves (tertiary) contained 1.3 per cent or approximately the normal percentage of the dry weight for unfumigated leaves. A positive correlation, therefore, exists between large leaf size, high internal-external surface ratios, large sulphur dioxide absorption, and high degree of leaf injury.

An analysis of variance shows that the difference between the internal surface ratio in the intervacular

cular interval and in the region of the major lateral veins is significant. Hill and Thomas (1933) noted that not even large leaves were injured near the veins. This observation correlates positively with the significant reduction in the internal exposed surface along the major veins of large leaves.

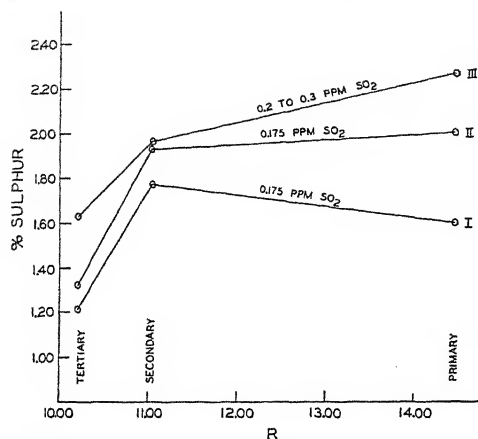


Fig. 23. The percentage sulphur found in primary, secondary, and tertiary leaves by Thomas and Hill (1937) plotted against the internal-external surface ratios (R) of primary ($R=14.52$), secondary ($R=10.94$), and tertiary ($R=10.34$) leaves. The concentration of SO_2 to which the leaves were subjected was 0.175, 0.175, and 0.2–0.3 ppm. in curves I, II, and III respectively. Note that the curves indicate that in this low concentration of gas the internal surface limits absorption of the gas principally in the tertiary leaves.

It is possible, however, that all the cellular material (mesophyll) in the vein islet acts as a unit, since translocation of absorbed and oxidized toxic gas from the mesophyll will depend upon the minor vein which surrounds it. The area of the vein islet in large leaves is significantly larger than that in small leaves, as calculated by the method of analysis of variance. In the average large leaf the mean internal-exposed surface of the mesophyll is 763,979 square microns in the average vein islet. For the average small leaf vein islet the mean internal exposed surface of the mesophyll is 382,406 square microns. The average cellular volume in the intervacular interval of large leaves is 3,213,000 cubic microns, and in small leaves the average cellular volume in the intervacular interval is 1,383,000 cubic microns. In the large leaves the ratio of the internal exposed surface for the intervacular interval to the volume of mesophyll cellular material is 0.238; in small leaves it is 0.240. Since these ratios are not significantly different, the ratio of the internal-exposed surface per vein islet, and the minor vein absorbing area or minor vein volume must be the effectual unit. The former in large leaves is 23.7, in small leaves, 18.7; while the latter is 3.22 for large leaves and 3.60 for small leaves. Clearly, if among other factors morphological factors affect absorption and

injury by SO_2 , the important factor must be the ratio of internal exposed surface of the vein islet and the area of the minor vein absorbing surface surrounding the islet.

The positive correlation of the internal-external exposed surface ratio with SO_2 absorption is shown in figure 23, in which analysis of primary, secondary, and tertiary leaves for sulphur by Thomas and Hill (1937) has been plotted against our measurements of internal surface ratios of primary, secondary, and tertiary leaves.

Thomas and Hill (1935) have shown the absorption value of sulphur dioxide by alfalfa, x , to be equal to tAC , where t equals time of fumigation (duration), C equals concentration of sulphur dioxide in the air, and A equals an absorption factor which is a measure of the activity of the plant. The value of A was determined under various conditions of light intensity, relative humidity, sulphur dioxide concentration, time of exposure, maturity of plants, succession of crops, seasons, and years (1928–1931). The ratio of the maximum absorption factor to the minimum is 21.6, while that of the largest internal-external surface ratio per sample area to the smallest is 2.08, and the ratio of the internal exposed surface per unit of minor vein area for large/small leaves is only 1.26. The ratio for the absorption factor is nearly ten times that for the internal-external surface ratio and over seventeen times the internal exposed surface per unit minor vein area for large and small leaves. The wide variation in the absorption coefficient A as compared with the variation in the internal-external surface ratio calculated to any base, shows that other factors in aggregate have a much greater effect on sulphur dioxide absorption under the large variety of conditions in which fumigations have been made. Wells (1917), for instance, has pointed out that plants are five to six times as resistant at night and suggests this is due to closure of the stomata. Conditions which would affect closure during the day might likewise make large increases in the resistance to SO_2 .

SUMMARY

Measurements of alfalfa leaves showed that areas of late primary, early primary, secondary, tertiary, and quaternary leaves were, on the average, successively smaller. The leaf thickness, upper and lower epidermal thicknesses, palisade and sponge thicknesses, midrib, major-vein, and minor-vein diameters were, in general, greater in the primary leaves and successively smaller in secondary, tertiary, and quaternary leaves. When the leaves were divided into two classes, large (those larger than 1 centimeter in area) and small (those smaller than 1 centimeter in area), the large leaves were found to have greater mean leaf thickness, upper and lower epidermal thickness, palisade and sponge thickness, and greater mean midrib, major-vein, and minor-vein diameters.

The internal-external surface ratios calculated for the intervacular interval, for the region of the major veins, and for the region of the minor veins were successively smaller for early primary, late primary, secondary, tertiary, and quaternary leaves. For any one of these classes of leaves, the internal-external surface ratio was largest in the intervacular interval, intermediate in the region of the minor veins, and smallest in the region of the major veins. When the leaves were grouped as large and small, the internal-external surface ratio was larger in the large leaves.

Volume of intercellular space was largest in early primary leaves, successively smaller in late primary, secondary, and tertiary leaves, and smallest in quaternary leaves. When the leaves were grouped as large and small, the large leaves had the greater volume of intercellular space.

The length and width of vein islets and of epidermal cells were greater in large leaves than in small leaves.

Stomatal density was lower in large leaves than in small leaves, but the stomatal pores were larger in the large leaves.

Leaf area was positively, moderately, and significantly correlated with epidermal, palisade, and leaf thickness, with major- and minor-vein diameter, and with internal-external surface ratio; the correlation coefficient was approximately $+0.5$. Leaf area was negatively, moderately, and significantly correlated with sponge-cell area (determined in tangential section), and the correlation coefficient was approximately -0.5 . Mesophyll thickness and internal-external surface ratio were positively, highly, and significantly correlated; and the correlation coefficient was $+0.9$, which suggests the possibility of using mesophyll thickness in estimating internal-external surface ratios from regression equations.

Large internal-external surface ratios, large volumes of intercellular space, large stomatal pores and large vein islet areas are positively correlated with large leaf size and reported greater SO_2 injury in large leaves.

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SHRINKAGE AND CELL WALL STRUCTURE OF COTTON FIBERS¹

Earl E. Berkley

THE SHRINKAGE of natural fibers such as cotton, is dependent upon the fine structure of the fiber and its physical condition. The structure, as variously described in the literature, is still debatable but the physical condition of the fibers can be controlled within reasonable limits. A study of the shrinkage resulting from a reduction in moisture content may aid in the determination of the fundamental structure of the fiber.

The microscopic structure of the cotton fiber has been described by Balls (1923), Denham (1923), Anderson and Kerr (1938), Hock, Ramsay and Harris (1941), and others. The fine structure as determined by x-ray diffraction has been described by Berkley (1939). Certain phases of the literature dealing with cellulose and cell wall structure in general have been reviewed recently by Bailey (1938, 1939), Kratky (1940), Mark (1940) and Nickerson (1940), and the need for studies in cotton fiber character were discussed by Conrad and Webb (1935), so no extensive review will be attempted here.

Briefly, the cotton fiber is a single cell and contains a primary wall and a secondary thickening as described by Kerr and Bailey (1934) for other plant fibers. The primary wall is the first formed layer and constitutes the thin sheath, approximately $0.5\ \mu$ in thickness, on the outer surface of the mature cotton fiber. The crystalline cellulose is arranged at an angle of 70 to 80 degrees to the fiber axis (Balls, 1923; Anderson and Kerr, 1938).

The secondary wall is laid down on the inside of the primary wall next to the lumen. The crystalline cellulose of the secondary thickening lies in a spiral in the fiber, direction of which changes frequently from an S to a Z spiral, or vice versa. According to Anderson and Kerr (1938), the direction of the spiral of the first layer is usually opposite that of the succeeding layers, all of which follow in general a given pattern. The pitch of the spiral, that is, the angle between the long axis of the fibrils and that of the fiber, varies with the variety of cotton and the climatic condition under which it is grown (Berkley, 1941; Kerr, 1941; Barre and Berkley, 1942).

The samples used in these studies varied in age from ten to thirty days after bloom and represent either the primary wall only, or both the primary and the secondary walls as indicated.

The shrinkage of "green" moist cotton fibers as a result of drying has received little attention in connection with the studies of quality of raw cotton and

other natural fibers. Collins (1930) discussed the swelling and shrinkage of air-dry mercerized cotton with changes in moisture content, but the results of his studies on modified cellulosic fibers are not comparable with those reported in this paper.

The term "shrinkage" as used here is intended to mean the contraction in length, breadth or volume of the cotton fiber, whereas the term "swelling" is used to indicate the expansion of the fiber in any one of these dimensions. The data are confined principally to the longitudinal shrinkage. By green condition is meant the wet fibers taken, without drying, from the bolls and kept thereafter, until used, in liquid preservatives.

MATERIALS AND METHODS.—Seven varieties representing two species of cotton, *Gossypium hirsutum* L. and *G. barbadense* L. were used in these studies. The samples were collected at Raleigh, North Carolina,² during the summers of 1937, 1939 and 1940. Flowers were tagged in the field and the samples collected at the desired ages. The young bolls, ten to thirty days old, were brought into the laboratory, opened immediately and the locks dropped into boiling water where they were allowed to remain for five to ten minutes. A number of measurements were made on fibers with no further treatment but the majority of the samples were preserved in sufficient 95 per cent alcohol to give approximately 70 per cent alcohol when combined with the water in the tissue.

The fibers on a given seed were paralleled by the method described by Berkley (1939) for the preparation of x-ray samples. This treatment consisted of combing the fibers under a stream of running water without removing them from the seed. A dissecting needle, passed through the seed and used to hold it while combing the fiber, was then stuck into one of two boards tacked together.³ It was inserted at such a height on the vertical board that the tips of the fibers matted on a small piece of soft filter paper placed on the board below. The fibers were permitted to drain until they settled around the seed and formed a relatively dense bundle. The distance between the bottom of the seed and the filter paper was then measured with a pair of dividers and a steel ruler graduated in hundredths of an inch. These bundles were then permitted to dry over night at room temperature. The distance between the bottom of the seed and the filter paper was again measured. From these two measurements the air-dry longitudinal shrinkage was calculated. A number of other

² Certain of the series of samples were collected and preserved by Dr. Thomas Kerr, Cytologist, Bureau of Plant Industry and North Carolina State Experiment Station and his staff at the North Carolina State College, Raleigh, North Carolina.

³ Two soft pine boards about three inches wide and four feet long were tacked together along the edges to form a trough or V. One board when lying on the table served as a base, the other stood vertical. The needles were placed in the vertical board.

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methods of preparation and measurement of the bundles were tried with less success.

The fibers which had coalesced into a hard, rigid bundle were then cut as near the seed and the filter paper as possible and remeasured. Half of the bundles were placed in water and allowed to soak over night. The other half were placed in a drying oven at about 105°C. and allowed to dry for about four hours, after which the oven-dry length was determined. The next day when the bundles from the water were measured, those from the oven were placed in water to soak over night and those from the water were oven-dried. In this way it was possible to measure the regain in length from both the air-dry and oven-dry condition to the water-wet condition.

CHEMICAL TREATMENTS.—The chemical treatments described here are in addition to the boiling water and preserving solution previously mentioned. A small number of samples were extracted with 95 per cent ethyl alcohol for about four hours to remove the waxes. A portion of these was measured for shrinkage without further treatment. Another portion was further treated to remove the pectic substance and then measured for shrinkage. Three methods were used to remove the pectic substance: first, by treating over night in 96 per cent acetic acid,

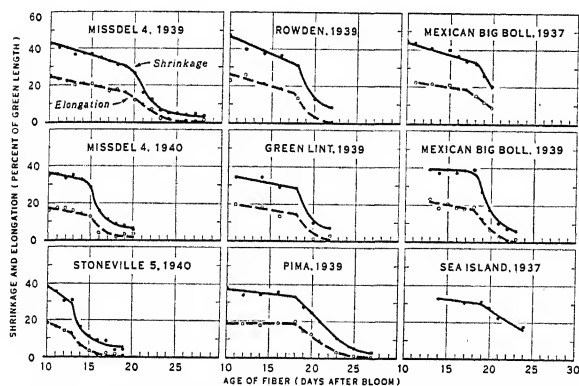


Fig. 1. Per cent shrinkage and elongation of cotton fibers representing various ages for seven different varieties of cotton. The recovery (broken line), is the percentage of the green length that the fiber elongated, after it was dried to the air-dry condition. For the most part the recovery was parallel with and amounted to more than 50 per cent of the shrinkage (solid line). Note the wide differences in slope and the position where the first break occurs in each series. The position of the break indicates the age at which secondary thickening was initiated in the fibers. Each plotted point represents the average of ten or more bundles.

washing and treating over night again in 2 per cent ammonia, according to the method described by Whistler, Martin and Harris (1940); second, by treating for several hours at 80° to 90°C. in ammonium oxalate or, third, by boiling for one hour or more in 1 per cent sodium hydroxide solution.

Approximately 50 per cent of the loss in length resulting from the removal of water by drying was

non-reversible when the fibers were submerged in water after drying. Caustic of mercerizing strength and cuprammonium solutions were used to swell the fiber further and to determine, if possible, the type of structure associated with this phenomenon in the primary walls of the cotton fiber.

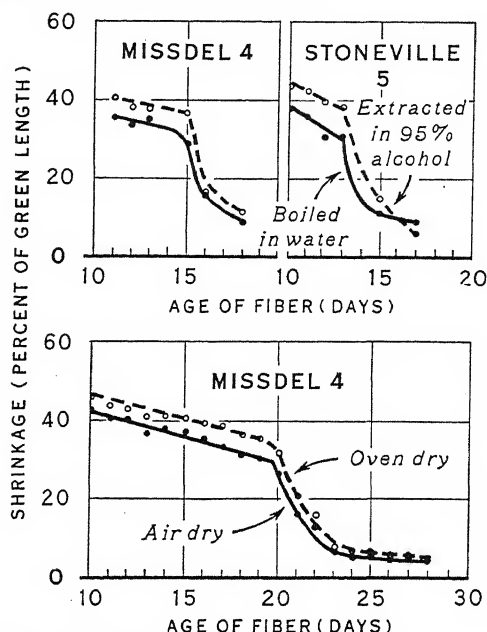


Fig. 2. Per cent shrinkage of cotton fibers of various ages after different treatments: upper charts show shrinkage after boiling in water (smooth lines) and after additional extraction with 95 per cent alcohol (broken line); lower chart shows shrinkage from alcohol preservation after air-drying (smooth line) and after additional oven-drying (broken line). Each plotted point represents an average of ten or more bundles.

Previously dried samples were swollen in water and then in 18 per cent sodium hydroxide or cuprammonium hydroxide solution⁴ and the percentage of elongation upon swelling was measured. As many as ten alternations of the swelling reagent and water were used on certain samples.

The treatments with 18 per cent sodium hydroxide were carried out at room temperature, at 80°C., and in an ice bath, or refrigerator. In all cases the caustic was washed out with distilled water at room temperature.

Difficulties in paralleling fibers.—Cotton fibers fold back and forth in the process of elongation in the growing boll and thus become kinky. In the early stages before secondary thickening appears, the fibers are very fragile. They tend to adhere to each other and break rather than straighten out when combed under a stream of running water. If they are first boiled in water for a few minutes the kinks gradually disappear and the surface of the fibers becomes slimy or gelatinous so that they may be paralleled with little difficulty.

⁴ Containing 30±0.2 g. copper and 165±1.0 g. ammonia per liter.

RESULTS.—*Shrinkage*.—When the fibers from ten-day-old cotton bolls were boiled in water, combed parallel and dried, they lost from 30 to 50 per cent of their original length (see fig. 1). During the following days, the lengthening fibers from successively older bolls showed gradually decreased shrinkage, up to the stage of secondary wall formation. When secondary thickening began, shrinkage rapidly fell

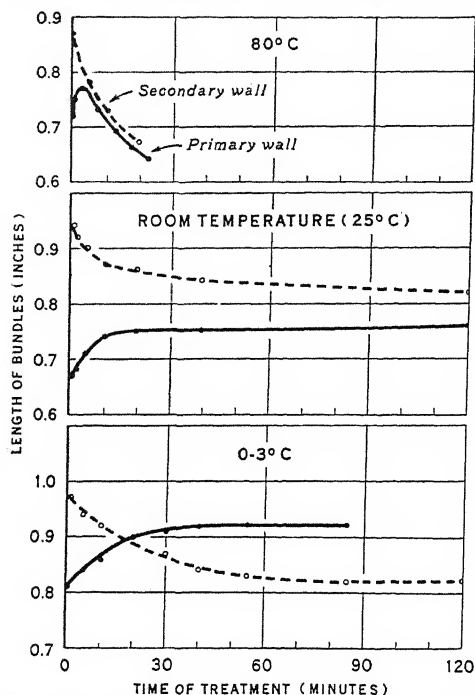


Fig. 3. The effects on cotton fiber length of 18 per cent sodium hydroxide at different temperatures. The solid lines represent the data for 18-day-old fibers and the broken lines, 25-day-old fibers.

off for a period of from two to five days, depending on the variety of cotton and weather conditions, after which only slight changes were observed.

This trend appears to be generally characteristic of the varieties of cotton studied, but the rate of change in the shrinkage during the period of elongation of the fibers varied appreciably. Figure 1 includes one series (Mexican Big Boll, 1939) in which the shrinkage showed little progressive change between the 13th and the 18th day after the flowers opened. On the 19th day, secondary wall was being laid down and the per cent shrinkage decreased appreciably, after which it continued to fall off more or less rapidly until the 22nd day.

During the primary wall stage, treatments such as extraction in hot 95 per cent ethyl alcohol to remove the waxes of the fiber increased the longitudinal shrinkage on the average about 4 per cent, as seen in figure 2. Further treatments to remove the pectic substances, such as with hot 0.5 per cent ammonium oxalate and 96 per cent acetic acid followed by 2 per cent ammonia, increased the shrinkage about 7 per cent more.

The fiber appears to become slimy on the surface during the boiling water treatment. It is difficult to determine what the boiling water does to the structure, since there are three systems: a wax, a pectin, and a cellulose system. The effect may be due to a disintegration of the pectic substances of the cotton fiber. If so, it is quite possible that an increased shrinkage of the fiber may be expected from this treatment.

In figure 2, the difference between the shrinkage when the bundles were dried to the air-dry, and subsequently to the oven-dry, condition may also be seen. Similar differences were observed in all series studied, but in order to save space the data have been omitted from this manuscript. The permanent or non-reversible shrinkage, as shown in figure 1, was also increased when the fibers were oven-dried. The air-dry fibers usually regained 50 per cent or more of their loss in length upon re-wetting in water, whereas the oven-dry samples regained a smaller percentage of their loss.

The ends of green cotton fibers, which contain primary wall only, were fastened and the cotton was allowed to dry. The fibers shrank in length as usual and in so doing the bundle ruptured. Before rupture occurred, there appeared to be a certain amount of flow in the cell wall substance, presumably in the amorphous portion, as shown by the drawing of the fiber bundle to a much smaller diameter. It is recognized that a certain percentage of the fibers could have ruptured during this process, thus reducing the diameter of the bundle, but no evidence of this was found.

Elongation due to swelling.—A certain percentage of the loss in length is recovered when green cotton fibers which have been previously dried are soaked in water. During the primary wall stage, 50 per cent or more of the air-dry shrinkage is reversible, as shown in figure 1, but after the secondary wall is formed this figure is greatly reduced. If the fiber bundles are dried to the oven-dry condition less than half of the loss at any stage is regained by subsequent soaking in water.

Previously dried bundles of cotton fiber which contained primary wall only increased in length, whereas those which contained secondary thickening decreased in length when submerged in 18 per cent sodium hydroxide, irrespective of temperature (fig. 3). The rate of change was increased by an increase in the temperature of the caustic. At 0 to 3°C., swelling reached a maximum in approximately one hour, although there were slight increases in certain samples upon prolonged treatment. At room temperature, the period required for the swelling to reach a maximum varied somewhat from sample to sample, but in general there was little increase after about twenty minutes (fig. 3). At 80°C. the increase of length of fibers containing primary walls only was very rapid but short lived. It was difficult to determine the extent of elongation as the fibers began to shrink in length within the first five minutes in the hot caustic. The bundles were rapidly dissolved so

that only a small residue in the form of a fine thread remained after about twenty minutes. Examination of the fibers under the microscope showed a reduction both in length and diameter. The samples with primary wall only, when treated at room temperature, began to decrease in length after about twenty-four hours, whereas those treated at 0° to 3° showed no signs of longitudinal contraction at the end of six weeks' treatment.

When bundles of fibers containing primary walls only were treated alternately in 18 per cent sodium hydroxide and water, the length changed rapidly. At 0° to 3°C. the bundles increased in length when washed in water but, when retreated with the caustic, they came back to approximately the same length they had assumed before. Water at room temperature was used in all cases regardless of the temperature of the caustic. There was little tendency for shrinkage in the samples treated in the cold caustic, but those treated at room temperature and particularly those treated at 80°C. showed a rapid decrease

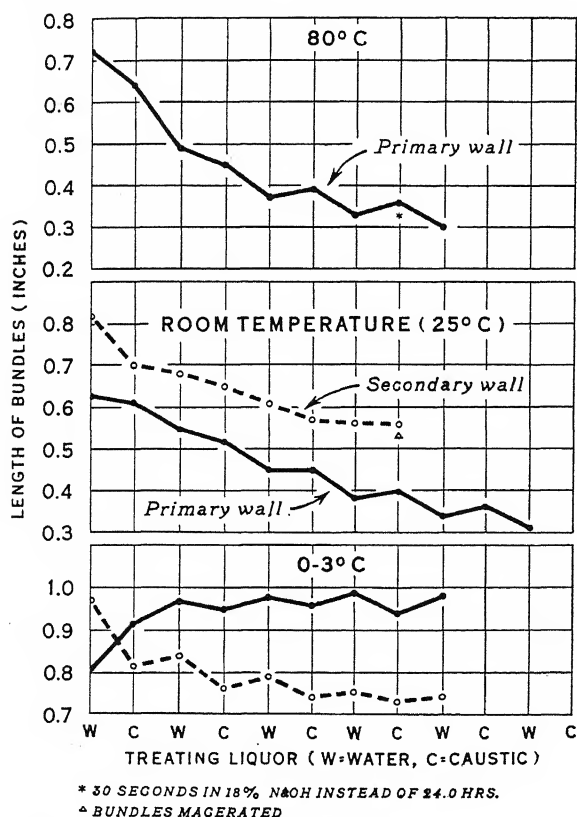


Fig. 4. The effects on cotton fiber lengths of alternate treatments with water and 18 per cent sodium hydroxide at different temperatures. The solid lines represent the data from the 18-day-old fibers; the broken lines, that from the 20- and 25-day-old fibers.

in length when washed in water. After the first two or three alternations, the bundles retained their length or increased slightly in the caustic but shrank appreciably in the water (fig. 4). Since the increase

of length in the caustic did not equal the decrease in length in the water, the bundles gradually lost up to 50 per cent or more of their lengths. Further treatments, no doubt, would have continued this reduction.

The bundles which contained secondary thickening showed a progressive change in length with alternate treatments of caustic and water at 0° to 3°C. and at room temperature but no tendency to shrink more rapidly in the water than in the caustic (fig. 4).

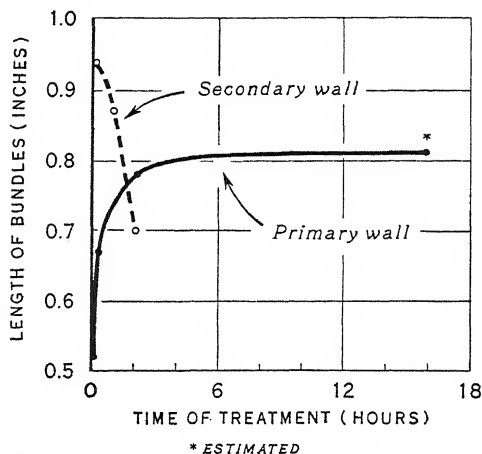


Fig. 5. The effects of cuprammonium hydroxide solution on the length of cotton fibers which had previously been dried. The solid line represents the data for 16-day-old fibers; the broken line for the 25-day-old fibers.

When bundles of dry fibers containing only primary walls were treated with a cuprammonium solution, they increased rapidly in length. The gain in length, as shown in figure 5, was from 0.52 to 0.81 inch, or approximately 56 per cent of its dry length, but in no case did the bundle swell to its original green or preserved length. On the other hand, a bundle of the fibers which contained secondary thickening shrank from 0.94 to 0.70 inch, about 26 per cent, in length before it became a jelly-like mass and dissolved. Those containing only primary walls remained intact until after they had been washed in water and treated again in cuprammonium hydroxide solution.

DISCUSSION.—It has been repeatedly demonstrated by the use of x-ray technique that the dimensions of the unit cell of the cellulose crystal do not change with changes of moisture content in the fiber. It is not likely, therefore, that the dimensions of the crystal will change under the same conditions. If this is true, the shrinkage of green cotton fibers, upon drying, must take place in the layers between the crystals. It remains only to demonstrate, then, whether the areas from which the water is removed are simply crystal faults or cracks between the crystals or whether they are areas of amorphous materials which, with the water, form a jell and serve as an adhesive material that binds the crystals together.

The water may wet the entire surface of a crystal and an individual molecule that does not form a part of the crystal lattice. Such molecules when existing in the amorphous state may be bound to other molecules or crystals at certain points but the major part of the long chain is wound in and out at random among the other molecules. The water covering its surface increases the volume of the mass, but the changes in volume with changes in moisture content are controlled more by the shape and size of the area of amorphous structure than by that of the molecules.

Kratky (1940) suggested that the enormous strength of cellulosic fibers could hardly be accounted for, unless there was some structure in the areas absorbing the water. This is especially true, since the dimensions of certain cotton fibers used by Collins (1930) in his studies changed as much as 48.6 per cent going from water-wet to zero per cent relative humidity on the basis of the area at zero per cent relative humidity. A layer of water thick enough to give the changes in area of mature fibers observed by Collins (1930) would undoubtedly weaken the fibers materially. It may be well to remember in this connection that the tensile strength of cotton fibers is usually increased with an increase in moisture content, rather than reduced. This can be explained on the basis of the presence of a somewhat plastic adhesive substance, such as an amorphous structure surrounding the crystals but it does not support the assumption that there are only cracks into which the moisture enters.

There has been considerable discussion in the literature concerning the size and shape of the crystallites or individual units of the cellulose crystal. Only a few of the suggestions will be mentioned here. Nägeli (1864) concluded, on the basis of the physical properties of cellulose, that the crystals or "micelles" were of sub-microscopic dimensions, and that water penetrated between them to cause swelling. Ritter (1935) observed needle-like strands which he termed "fusiform bodies," and Hock and Seifriz (1940) and Wergin (1938) stated that the fibrils could be broken down into smaller units.

Farr and Eckerson (1934) suggested that the cellulose was discontinuous in the form of ellipsoidal particles, each covered with a thin layer of pectic substance. The presence of such particles has not been confirmed and the existence of a pectic adhesive material surrounding the cellulose has been thoroughly disproved as shown by the work of Whistler, Martin, and Harris (1940), Howells (1941) and Heuser and Green (1941).

Bailey and Kerr (1935, 1937) suggested that, in the case of wood, both the cellulose and non-cellulose systems were continuous in the form of a net-like structure and that the units graded down to the limits of microscopic visibility. This theory was further amplified by Bailey (1938, 1939) and by Frey-Wyssling (1936, 1937, 1938a, 1938b).

Kratky and Mark (1937) working on regenerated cellulose, suggested that, instead of having discrete crystalline units, a given cellulose molecule may ex-

tend through a large number of crystals with the intermittent segments existing in the amorphous state.

The writer has been able to macerate in cuprammonium solution and phosphoric acid cotton fiber cell walls into fine needle-shaped units which grade down to the limits of microscopic visibility. Long slender lines which appear to be the light diffracted from small units which cannot be resolved can be seen as they separate from the cell and float away in the macerating fluids.

At present the best hypothesis appears to be that the cellulose crystals may or may not be sub-microscopic in size. They undoubtedly reach a length as great as that of the cellulose molecule which is thought to be as much as 1 to $1\frac{1}{2}\mu$ long, and they are surrounded by an amorphous matrix which, in the case of the secondary wall of the cotton fiber, at least, appears to be cellulose itself. The non-cellulose constituents of the primary wall, such as, for example, the waxes and the pectic substances, may or may not be crystalline, but they too are dispersed in this amorphous matrix and each may, in turn, exist as a continuous system.

Hock and Harris (1940) found that the pectic material of the primary wall of the cotton fiber formed a continuous membrane after they treated the fiber to remove all the cellulose. It has also been demonstrated that treatments to remove the pectic substances and the waxes do not reduce the tensile strength of cotton; therefore, the cellulose must be continuous. According to these interpretations it would appear that Farr and Eckerson (1934) were correct in their assumption that particles of cellulose were surrounded by an isotropic matrix, but they were apparently mistaken about the size and shape of the particles and the chemical nature of the adhesive substance.

In summarizing, the cellulose of the cotton fiber may be considered as existing both in the crystalline and the non-crystalline states as illustrated in figure 6. The crystals, like the fiber, are apparently many times longer than broad and may or may not be aggregated into bundles such as fibrils. The amorphous substance between these fine crystals is largely cellulose, and it contains an enormous quantity of water in the native green state, much of which is permanently lost upon the drying of the fiber. As the water disappears, the amorphous structure shrinks. Since the crystals are not all parallel, they may bridge to prevent the units from coming together and the amorphous structure ruptures to form air spaces in the tissue. These air spaces are probably irregular in shape and position, but it is indicated that they may be more numerous in certain areas of the fiber than in others. For example, the rapid swelling of the less dense portion of the daily growth ring of the cotton fiber, in contrast to the denser portion, as shown by Kerr (1937), may be due to its porous nature which permits the rapid penetration of the swelling agent.

With this picture in mind (see fig. 6) there are two possible explanations for the changes in shrinkage

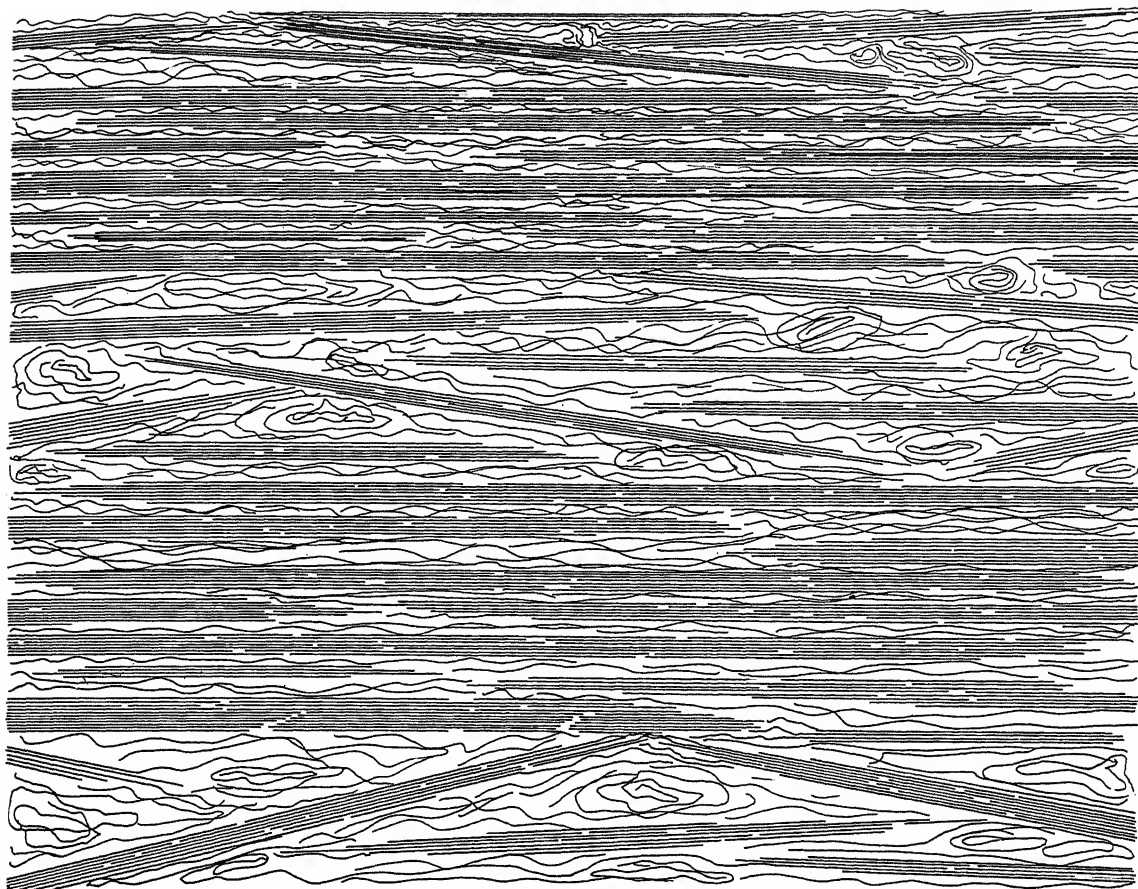


Fig. 6. A diagrammatic sketch of the fine structure of cellulose in cotton fibers. The parallel lines are intended to represent the crystalline and the wavy lines, the amorphous cellulose. Each group of parallel lines make up a crystallite, and each group of crystallites may be considered as representing a fibril.

of the primary wall of the cotton fiber with increasing age:

First, the orientation of the cellulose in relation to the major fiber axis has been observed by Anderson and Kerr (1938) to vary as the fiber elongates. Hock and Harris (1940) further observed that this variation was progressive and that the cellulose in the tip of the fiber was more nearly perpendicular with the fiber axis than that at the base.

Second, Anderson and Kerr (1938) have observed that the birefringence of the primary wall of the cotton fiber also increases with increased age. This may be due either to an increase in the degree of crystallization of the cellulose already present in the fiber wall or to the deposition of additional crystalline cellulose after the wall is formed. Another possibility is that there is more crystalline cellulose deposited in each of the successive portions of the wall as the fiber elongates.

Further evidence of the presence of an amorphous layer between the crystals is suggested from the permanent loss of length upon drying. Approximately 50 per cent of the loss in length of the young fibers, which contain primary walls only, is permanently

lost upon drying. This represents about one-fifth of the total length of the fiber. This loss could be accounted for on the assumption that the crystals are discrete and are separated only by cracks or faults, and that bonds form between certain of the crystals and thus prevent the re-entrance of the water. In such a case, it is difficult to understand why the swelling agents used in these studies, such as cuprammonium solution or 18 per cent sodium hydroxide, would not sever these bonds and permit the fiber to swell back to its original length. This does not take place as shown by the data, and the nature of the swelling observed would indicate that the presence of an amorphous structure between the crystal units is more likely.

It is easier to visualize that bonds form between individual cellulose molecules in the amorphous regions, thus preventing the full amount of swelling that would otherwise have taken place in the water, and, since the molecules are already dispersed to a certain degree, they would probably go into solution rather than swell appreciably in the cuprammonium solution and the mercerizing caustic.

The major portion of the primary wall of the cotton fiber appears to be made up principally of amorphous materials with crystalline cellulose and waxes dispersed more or less widely in them. So far as can be determined the pectic compounds and the waxes are largely confined to the primary wall. There may be some pectin on the surface of the lumen and some waxy material in the protoplasmic residues. The amorphous phase of the secondary thickening is less extensive than in the primary wall and appears to be composed of cellulose. The slight longitudinal shrinkage observed in the secondary thickening can be accounted for on the assumption that the cellulose crystals are many times longer than broad. They overlap in such a way that there is practically no shrinkage along their major axes. The shrinkage that is observed along the fiber axis is apparently that expected from the component of the lateral shrinkage due to the spiral of the cellulose in the fiber.

The loss of length of the fibers with primary wall only, in the mercerizing caustic at room temperature and above, needs further study before reliable conclusions can be drawn. It would appear from the shrinkage that the cell wall substance is highly soluble in the caustic. The soluble portion is probably the amorphous material. It has also been found that the crystalline phase of the cellulose shows up much better after an extraction in alkali and a wax solvent (Sisson, 1937; Berkley, 1939). This is due to the reduction of the amorphous halo and the wax lines in the patterns. It may be assumed from this that the amorphous phase is soluble in the alkali and that it makes up an appreciable part of the primary wall of the cotton fiber.

SUMMARY

Longitudinal shrinkage of cotton fibers, when dried from the green to the air-dry and the oven-dry conditions, was measured on seven varieties, representing two species of cotton (*Gossypium hirsutum* and *G. barbadense*), collected at frequent intervals beginning ten days after the flowers opened. At the age of ten days the air-dry shrinkage was about 35 to 40 per cent of the "green" moist length of the fiber.

From the age of about ten days to the time secondary thickening was initiated, the shrinkage gradually decreased. One series of samples from the Mexican Big Boll variety showed little or no reduction between the thirteenth and eighteenth days. Two other series from the same variety showed the usual decline in shrinkage.

After secondary thickening started, the shrinkage decreased rapidly for the first two to five days, after which it changed very little.

All varieties showed similar trends but the rate of change was greater in the upland varieties than in the Pima or Sea Island cottons.

There was an increase of about 4 per cent in the shrinkage when the waxes were removed.

There was an appreciable increase in the shrinkage after treating the fiber bundles to remove the pectic substances.

The increase in shrinkage upon the removal of the waxes and pectic substances indicates that these substances are dispersed in or interwoven with the cellulose of the cell wall.

There was a significant decrease in the per cent shrinkage when the fibers were dewaxed and then treated in boiling 1 per cent sodium hydroxide for one hour.

At 80°C. bundles of fibers containing primary wall only, treated in 18 per cent caustic, showed a rapid increase in length for about two minutes, after which they showed a marked shrinkage which was amplified by frequent washes in water.

At room temperature similar bundles of fibers, which contained primary walls only, showed an increase in length for a few minutes when treated in 18 per cent caustic, after which the length remained constant or decreased. Alternate treatments in water and caustic resulted in progressive shrinkage with no indication of a limit to the reduction in length to be expected.

At 0° to 3°C. similar bundles of fibers increased in length when treated in 18 per cent sodium hydroxide. They increased further in length upon submerging in water at room temperature. Alternate treatments of this type caused little additional change, but what change did occur was in the nature of an increase rather than a decrease in length.

Bundles of fibers which contained secondary thickening decreased in length when submerged in 18 per cent sodium hydroxide regardless of the temperature. The rate of change increased with a rise in temperature.

Bundles of fibers which contained primary walls only elongated when treated in cuprammonium solution. The increase in length was as much as 66 per cent of the dry length of bundles. These bundles expanded very little laterally until after they were washed in water and resubmerged in the cuprammonium solution.

Bundles of fibers which contained secondary thickening shrank in length when submerged in cuprammonium solution. They swelled laterally and soon ruptured to form a jelly.

In no instance did the bundles once dried to the air-dry or oven-dry condition swell to their original green length, regardless of the swelling agent or treatment.

These results confirm the microscopic and x-ray studies which show that the crystalline cellulose of the primary wall lies transverse to the long axis of the fiber or in a low spiral around the fiber.

They also indicate that only a small percentage of the primary wall is crystalline cellulose.

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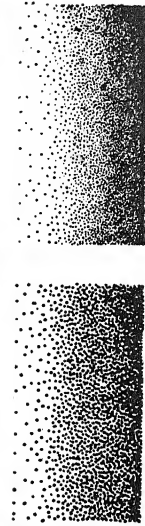
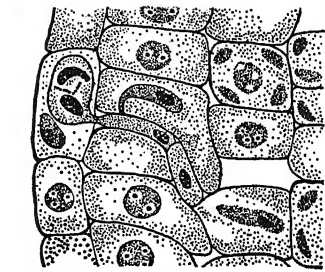
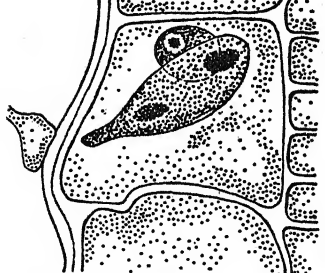
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DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

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Note that thin black lines hold up fairly well in reduction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. Keep the shading rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

CHANGES IN THE METABOLISM OF WHEAT LEAVES INDUCED BY INFECTION WITH POWDERY MILDEW¹

Paul J. Allen

THE POWDERY mildews infecting wheat are parasites whose nutritional requirements are extremely exacting. These requirements are satisfactorily met in wheat leaves which are adequately supplied with carbohydrate (Trelease and Trelease, 1929), but not in leaves whose carbohydrate supply is deficient. It is, therefore, probable that the development of mildew is closely related to the carbohydrate metabolism of its host, and that a knowledge of this metabolism may throw some light on the nature of the host contributions of importance in the development of the parasite. A study has, therefore, been made of some aspects of the carbohydrate metabolism of wheat leaves as they are influenced by mildew and as they in turn influence the development of mildew. Respiration, respiratory quotients, photosynthesis and chlorophyll content, and glucose, sucrose, and starch have been measured at frequent intervals from the time of infection until the death of the leaves. The peculiar changes leading to the formation of green islands have also been followed, and are discussed in this paper.

MATERIALS AND METHODS.—*Growth of the plants.*—All of the wheat plants used in these experiments were grown in full strength Hoagland's solution in two-quart jars. The early series were grown in the greenhouse, the later series in a control chamber.² In the latter the temperature was kept at $19 \pm 2^\circ\text{C}$., the humidity at 70 ± 10 per cent, and the plants were illuminated for sixteen hours daily with a circle of eleven 30-watt daylight fluorescent lamps supplemented with seven red lamps. These gave an intensity of approximately 150 foot candles. For series F a somewhat higher intensity was obtained using six additional daylight lamps. In table 1 is presented a summary of the culture conditions and the kind of seed used in the different series.

The strain of *Erysiphe graminis tritici* used in this study is highly pathogenic for Axminster and, according to Mains' classification (1933), is, therefore, strain number two. Inoculations were made by painting spores on one side of the leaf with a dry camel's hair brush. In this way the density of inoculation could be roughly controlled, though never with great accuracy. With heavy inoculations differences in the actual number of colonies per cm.² be-

came less significant, as shown by the even tenor of changes in heavily inoculated leaves.

Handling of experimental material.—Manometric experiments were always begun between 8.30 and 9.30 a.m. and required a total of four to five hours to complete. The first foliage leaf, which has been dealt with exclusively in these experiments, was removed from the seedling, one to two centimeters of the base and tip were discarded, and the rest of the leaf was cut into two or three pieces in order to fit the experimental vessels. The error involved due to loss of water while weighing was minimized by standardizing the weighing procedure, but may still have been great enough to account for some of the variability experienced throughout these investigations. In series A_c and A the chlorophyll content was determined on the same piece of leaf whose respiration and photosynthesis were measured. In series D and E one of the two or three pieces of each leaf was cut longitudinally and the two halves used respectively for measurements of photosynthesis and chlorophyll content. The measurements of respiration were made, using one of these halves together with the remainder of the leaf. Usually the remainder of the leaf was removed before measuring photosynthesis, in order that the rate of gas exchange should be suitable.

Determination of chlorophyll content.—The chlorophyll content was determined by a method similar to that of Emerson (1929). After boiling for two minutes in distilled water, weighed samples were extracted until white in commercial methyl alcohol. The per cent transmission of the resulting solutions was measured at 670 m μ with a Bausch and Lomb spectrophotometer. The density, D, in arbitrary units, was obtained from the transmission, T, from the relation $D = \log 1/T$. The values of D were converted to moles and expressed as the chlorophyll content of one milligram of fresh leaf tissue, using a conversion factor obtained by simultaneously determining the transmission and the Mg content of a methyl alcohol extract of two kilograms of seedling wheat leaves. The extract was purified of inorganic Mg by transferring to toluene and washing repeatedly with distilled water. Determinations of this factor were made on two separate samples of wheat and yielded values which differed from each other by only 5 per cent. The two were therefore averaged, giving a conversion factor of 23×10^{-9} , which is the moles of chlorophyll per ml. which would give an absorption of 1.0.

Manometric technique.—All measurements of gas exchange were made with a Fenn microvolumeter at 22°C . The experimental conditions were similar to those described previously (Allen and Goddard,

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Contribution from the Department of Botany, University of California, Berkeley, California.

I wish to express my thanks to Prof. A. R. Davis for his generous support of this research project, and to Dr. L. C. Marshall for advice and help in obtaining uniform light intensity.

² A description of this control chamber will be published at a later date.

1938b). The actual temperature control was $\pm 0.002^\circ$ in the dark and $\pm 0.005^\circ$ when the lights were on. The pieces of leaf were floated on 2 ml. of phosphate buffer at pH 6.0 and their O_2 consumption measured with 10 per cent KOH in the side arm. CO_2 production was measured with the same tissues by determining the difference between the change of gas volume with and without KOH in the side arm.

After the respiratory measurements were made, the air in the experimental vessels was replaced with an atmosphere containing 5 per cent CO_2 and 95 per cent N_2 , and photosynthesis was measured by a method similar to that of Warburg (1919), assuming a photosynthetic quotient of 1.0.³ The source of illumination was a bank of 200-watt frosted Mazda incandescent bulbs, whose light entered the bath horizontally through a window in the front and was reflected through an angle of 90° by a glass mirror hung beneath the experimental vessels. The intensity was varied by moving the bank away from the front of the bath. The actual intensities at the base of the vessels were measured with a Weston photronic cell. For measurements of photosynthesis in the range of limiting light intensities, the power supply to these lamps was regulated by means of four RCA Radio-tron ballast tubes arranged in parallel with each other and in series with the lamps. With this circuit a uniform output of 116 volts and 8.20 amperes was obtained over an input range of 150 to 160 volts.

Carbohydrate determinations.—Sugar and starch analyses were made by the ferricyanide-ceric sulfate titration method, using setopaline C as indicator. In the preparation of extracts the procedure described by Hassid (1937) was followed. After removing the alcohol from the sugar extracts and clarifying, the solutions were made up to 20 ml., half of which was immediately analyzed for reducing sugars. The remainder was inverted for $1\frac{1}{2}$ hours at $38^\circ C$. with invertase and the total sugars then determined. The reducing sugars were calculated as glucose in both cases, using a conversion factor of 39.15 mg. glucose equivalent to 1 ml. of molar ceric sulfate.

For starch determinations, the dried residue left after extracting the sugars was ground to a fine powder, heated in distilled water on a steam bath for one hour, and the suspension then made up to volume. An aliquot was removed for a blank, the remainder was hydrolyzed for $1\frac{1}{2}$ hours with salivary amylase, and the suspension clarified and filtered. To the blank aliquot was added the proper amount of salivary amylase which had been heated to $100^\circ C$. for three minutes. Starch was calculated as maltose, using a conversion factor 1.2 times the glucose factor (Giragosintz, Davidson, and Kirk, 1936).

The I-KI test for starch was used in determining the distribution of starch in infected leaves after removal of the chlorophyll. Although the appearance of a discoloration with I-KI is not incontrovertible evidence of the presence of starch, it was interpreted

³ The photosynthetic quotient of wheat leaves was determined in four cases, and yielded values of .98, .94, .90, and .90.

as such in these leaves because of the bluish-black hue of the discoloration, and because microscopic examination showed that much of the color was in the plastids. The mildew spores, on the other hand, stained a reddish brown.

Method of handling data.—The variability in the experimental material is in itself frequently of interest, and is, therefore, given in full in the following figures. Each curve is drawn through the daily average of all determinations on similar leaves, and through each point on the curve a vertical line indicates the range of individual determinations.

Since the ratio of photosynthesis to respiration in healthy leaves is so high (about 100:1), no correction for respiration has been made in the normal photosynthesis curves. In the case of infected leaves, however, this ratio becomes as low as 3:1 and is, therefore, of much greater significance. Consequently, a respiratory correction has been introduced in the determination of all values for photosynthesis of infected leaves.

EXPERIMENTAL RESULTS.—Respiration.—The experiments of Pratt (1938) and of Allen and Goddard (1938a, b) showed that the respiratory activity of wheat leaves infected with powdery mildew is considerably higher than that of normal leaves, and demonstrated clearly that the increased respiration resides largely in the uninvaded cells of the host. From these experiments, the latter authors concluded that toxic substances are produced by the mildew, diffuse across into the underlying host cells, and there initiate metabolic changes which result in an increase in both respiration and fermentation. Their curves for the time course of respiratory changes were obtained from leaves of soil grown seedlings inoculated by dusting with mildew conidia and indicate that the initial drop from the high respiratory rate following infection is succeeded by a secondary rise. For the experiments reported in this paper plants were grown in water culture solution and inoculated more uniformly by painting the spores on one side of each leaf. If leaves are heavily inoculated in this way, so that the entire surface is soon covered with mycelium, and there is no opportunity for secondary infection, the respiratory increase reaches a maximum seven to ten days after inoculation and then declines to a level near the normal, which is maintained until the death of the diseased leaves. In figures 1 and 2 the course of these changes is shown for leaves grown, respectively, in the greenhouse in summer (series A), and in the control chamber (series D). If, on the other hand, similar leaves are lightly inoculated (20–100 colonies/cm.²), so that there is opportunity for reinfection, a secondary rise in respiration occurs before the initial decline has progressed very far (fig. 3). The respiratory changes in this series were correlated with the development of secondary infection. Nine or ten days after inoculation, the primary colonies were sporulating heavily. These spores were shed and occupied the uninfected portions of the leaf, and at the time of the secondary maximum, six

or seven days later, many new colonies which were sufficiently mature to exert a strong effect on respiration had appeared all over the leaves.

These observations offer an adequate explanation for the secondary rise reported by Allen and Goddard in 1938. They also show that portions of diseased leaves which are not actually occupied by mildew colonies retain for some time their ability to undergo a respiratory increase. This is in accord with the observation that uninfected segments of diseased leaves show no increase in respiratory rate (Allen and Goddard, 1938b), and offers additional evidence for the restricted spread of mildew toxins.

Wheat plants grown in the greenhouse in winter are much less vigorous than those grown under more adequate light conditions. Associated with this unhealthy condition of the host plant is a decrease in the response to heavy inoculation with mildew. A relatively small number of colonies develop, so that the leaves give the appearance at first of having been lightly inoculated. The curve in figure 4 shows the small increase in respiration in such leaves. A maximum is reached earlier than in more vigorous plants, but the infected leaves survive about the same length of time as healthy leaves which have been heavily inoculated.

An increased respiration in other plants infected with obligate parasites has been found by Reed and Crabill (1915), Iljin (1923), Maresquelles (1928), Kourssanov (1928a), Yarwood (1934a, b), and other investigators.

The respiratory quotient.—In the preceding discussion only measurements of O_2 consumption have been reported. These measurements give no clue as to the nature of the substrate which is being oxidized. In two series the CO_2 production and O_2 consumption were, therefore, measured consecutively on the same tissue samples, and the R.Q.'s were calculated. The curves of figures 5 and 6 show the changes in R.Q. in the lightly infected leaves of series E and the heavily infected leaves of series D. (For the O_2 consumption in these two series see figures 2 and 3.) In series D the R.Q. is greater than 0.9 until after the respiratory maximum is reached, and drops to a fairly constant value around 0.85 after the decline in O_2 consumption sets in. The high R.Q. on the eighth day after inoculation occurs at the time of the first decline in respiration and is probably significant. In the lightly infected leaves of series E the R.Q. is high at the time of inoculation. This high R.Q. appears to be characteristic of young leaves. Before the respiratory rate has risen very far, the R.Q. reaches a value of about 1.05 which is maintained, with minor fluctuations, until respiration is at a maximum. Thereafter it decreases slightly, but still remains fairly close to 1.0.

These figures indicate that carbohydrate is the primary substrate being oxidized, at least during the phase of increasing respiration. In heavily infected leaves there is evidence for a definite change to the oxidation of additional substrates as the respiratory maximum is reached. But in lightly in-

fect leaves carbohydrate seems to be the primary substrate even after the first maximum in respiration is passed. The maintenance of an R.Q. close to 1.0 is associated with a continued high respiratory rate (cf. fig. 3).

The respiratory changes bear an interesting relation to the growth of the parasite. The major part of the vegetative growth of mildew colonies takes

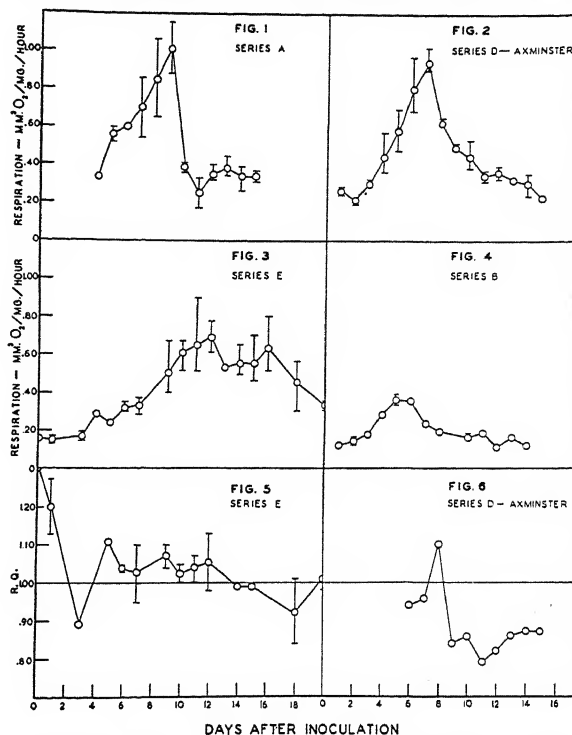


Fig. 1-6.—Fig. 1-4. Changes in respiration in plants inoculated with mildew.—Fig. 1. Marquis plants grown in the greenhouse in August, heavily inoculated when ten days old.—Fig. 2. Axminster grown in control chamber, heavily inoculated when twelve days old.—Fig. 3. Pure line Marquis grown in control chamber, lightly inoculated when twelve days old.—Fig. 4. Marquis grown in the greenhouse in December, heavily inoculated when twelve days old.—Fig. 5-6. Changes in respiratory quotient of the leaves of series E and D, respectively.

place during the phase of increasing respiration, and an arrest in their growth appears concurrently with an arrest in the respiratory increase and a change to the oxidation of non-carbohydrate substrates. This is true whether the leaves are heavily or lightly inoculated. Since the arrest occurs earlier in heavily infected leaves, the individual colonies on such leaves never become as large as they do on lightly infected ones.

Photosynthesis.—The influence of parasitic infection on the photosynthesis of higher plants has been studied by several investigators, with diverse results. Kourssanov (1928b) measured the photosynthetic activity of wheat infected with *Ustilago tritici* and found it to be slightly higher than that of normal wheat. Iljin (1923), on the other hand, found

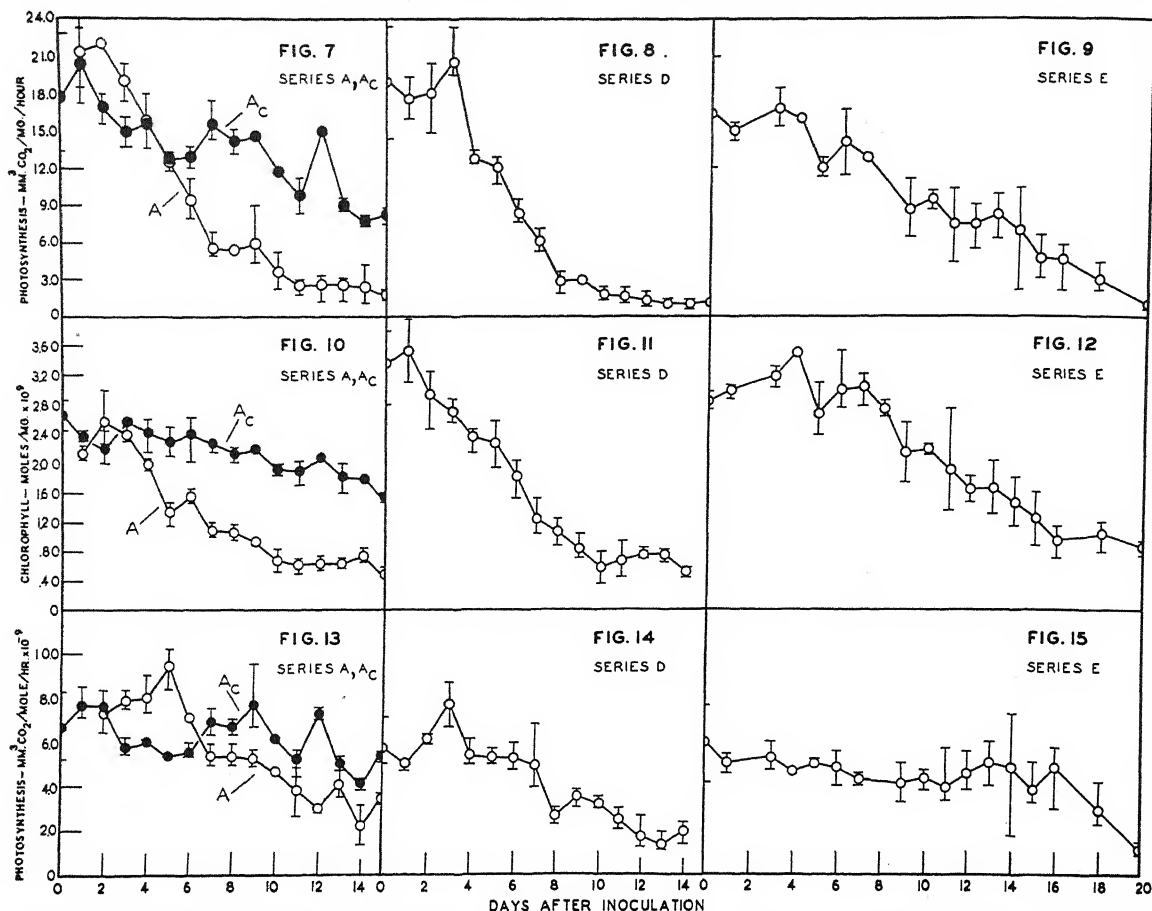


Fig. 7-15.—Fig. 7-9. Changes in photosynthesis/mg. fresh weight of normal (series A_c) and infected (series A, D, E) wheat leaves.—Fig. 7A_c. Photosynthesis of healthy Marquis between ten and twenty-five days old, grown in the greenhouse in August.—Fig. 7A. Marquis grown in the greenhouse in August, heavily inoculated when ten days old.—Fig. 8. Axminster grown in control chamber and heavily inoculated when twelve days old.—Fig. 9. Pure line of Marquis grown in control chamber, lightly inoculated when twelve days old.—Fig. 10-12. Changes in chlorophyll content of normal (series A_c) and infected (series A, D, E) wheat leaves.—Fig. 10A_c. Same as figure 7A_c.—Fig. 10A. Same as figure 7A.—Fig. 11. Same as figure 8.—Fig. 12. Same as figure 9.—Fig. 13-15. Changes in photosynthetic efficiency in normal (series A_c) and infected (series A, D, and E) wheat leaves.—Fig. 13A_c. Same as figure 7A_c.—Fig. 13A. Same as figure 7A.—Fig. 14. Same as figure 8.—Fig. 15. Same as figure 9.

a decrease in the photosynthesis of sunflower leaves infected with *Puccinia Helianthi* and of clover leaves infected with *Erysiphe Marthii*. Similarly, Reed and Crabill (1915) found that the photosynthesis of apple leaves infected with *Gymnosporangium Juniperi-virginianae* was lower than normal.

In order to make a comparison of the photosynthetic ability of the cells of diseased and healthy plants, it is necessary that the measurements be made when no external factors are limiting the rate. Preliminary experiments were, therefore, carried out to determine the conditions of CO₂ concentration, stomatal opening, and light intensity which would allow maximum photosynthesis of wheat leaves. It was found that an increase of CO₂ above 2 per cent by volume had no effect on the photosynthesis of the leaf pieces used in these experiments. The size of the stomatal aperture under differ-

ent conditions was determined with a reflection objective attached to a compound microscope. In leaves which had just been removed from the seedlings the stomata usually appeared closed; after an hour's exposure to strong light in the saturated atmosphere of the experimental vessels, the stomata were wide open; if exposed to lower illumination they opened more slowly and never as widely. Yet the rate of photosynthesis at a given light intensity did not change while these changes in stomatal aperture were going on. It was, therefore, considered that stomatal opening would not enter as a factor limiting the supply of CO₂ under the conditions of these experiments. Photosynthesis-light intensity curves were obtained, and it was found that the rate of CO₂ reduction was proportional to light intensity up to about 500 foot candles, and between 500 and

600 foot candles the curves gradually sloped off to a plateau.

It is interesting to compare the behavior of the isolated leaf pieces used in these experiments with that of entire plants of the same variety of wheat. Hoover *et al.* (1933), using seedlings of Marquis wheat, found that photosynthesis was dependent on light intensity even up to 2,000 foot candles when the CO₂ concentration was high (.15 per cent). Since photosynthesis was expressed in arbitrary units, it is not possible to compare their maximum values with those for leaf pieces. Brackett (1935), however, states that the photosynthesis respiration ratio was 1:35, while this ratio in the present experiments was about 1:100. Assuming a similar rate of respiration in the two cases, these figures indicate that the maximal rate of photosynthesis of leaf pieces at 600 foot candles is nearly three times as great as that of entire seedlings at 2,000 foot candles. The difference is probably due to the fact that much thick tissue and tissue relatively poor in chlorophyll is included in the entire plant and to the shading of tissues in the stem and near the leaf bases. The behavior of the isolated leaf is much more like that of unicellular green plants, such as *Chlorella* (Warburg, 1919) and the diatom *Nitzschia* (Barker, 1935).

Photosynthesis per milligram fresh leaf.—The photosynthesis of normal leaves declines gradually and irregularly with age (fig. 7A_c). Although there are large diurnal fluctuations, the values for a given day agree with each other quite closely. This is equally true for plants grown in the greenhouse or in the control chamber. The origin of these fluctuations is, therefore, not in changes in the environment but in internal changes.

If leaves are heavily inoculated with mildew, their ability to carry on photosynthesis under conditions of light saturation soon begins to decrease. In figure 7A the photosynthesis of Marquis leaves grown in the greenhouse is plotted against age of infection. It drops rapidly from a high value of 21.0 mm.³/mg./hr. two days after inoculation to a value of about 6.0 on the seventh day. Subsequently there is a continued slow decline until the death of the leaves, about two weeks after inoculation. The response of Axminster wheat grown in the control chamber is very similar (fig. 8), except that the decline does not start until three days after inoculation and is not checked until eight days after inoculation, when it has reached a value of about 3.0 mm.³/mg./hr.

If leaves are lightly inoculated, the influence of the mildew appears more gradually, and the decline in photosynthesis continues for a longer period than in heavily infected leaves (fig. 9). Before the leaves die, however, photosynthesis becomes just as low as in more severely infected leaves. The variability in this series is large because of the greater differences between the actual number of colonies on each leaf.

Since the above measurements were made under conditions of light and CO₂ saturation, they repre-

sent changes in the activity of the Blackman reactions. The effects of the mildew may, therefore, be extended to include a more or less gradual impairment of the activity of the dark reactions.

Chlorophyll content.—The chlorophyll content of normal leaves is, like the rate of photosynthesis, a function of the age of the seedling. Figure 10A_c shows the course of changes in chlorophyll of normal leaves of Marquis grown in the greenhouse. These changes follow a more uniform trend than the changes in photosynthesis. Heavy inoculation with mildew results in a rapid destruction of chlorophyll (fig. 10A and 11). The curves for chlorophyll content are quite similar in their general features to the curves for photosynthesis. This is also the case for lightly inoculated leaves (fig. 12), whose chlorophyll content, like their photosynthetic rate, drops more slowly. There are, nevertheless, certain obvious differences between the curves for chlorophyll content and those for photosynthesis. In series A there is an increase in chlorophyll between the fifth and sixth days after inoculation but no corresponding increase in photosynthesis. Since photosynthesis and chlorophyll content were both determined for the same leaf pieces, this difference is not due to the variability of different samples. During the last few days of each series there is a slight increase in chlorophyll but no corresponding increase in photosynthesis. This difference is particularly clear in series D.

Photosynthesis per mole of chlorophyll.—From the values for photosynthesis and chlorophyll content the photosynthetic efficiency or the photosynthesis per mole of chlorophyll may be calculated. If changes in the rate of photosynthesis are determined by changes in chlorophyll content alone, the photosynthetic efficiency will remain constant. If such changes represent alterations in other parts of the photosynthetic mechanism, the photosynthetic efficiency may change. The destruction of chlorophyll which is not functioning at its maximum capacity (because of the slowness of the dark reactions) will cause an increase in photosynthetic efficiency. Or if the inactivation of other parts of the photosynthetic mechanism proceeds more rapidly than the destruction of chlorophyll, there may be a decrease in photosynthetic efficiency.

The value for photosynthetic efficiency have been calculated for each leaf used in series A_c, A, D, and E. The individual values were averaged, and the resulting curves are plotted in figures 13 to 15. The changes in photosynthetic efficiency of normal leaves show a striking parallelism with the changes in photosynthesis of the same leaves (cf. fig. 7). The fluctuations in photosynthesis of these leaves do not, therefore, depend on variations in chlorophyll, but on variations in other parts of the photosynthetic mechanism. Diseased leaves, on the other hand, present a different picture. In the heavily infected leaves of series A and D there is an increase in the photosynthetic efficiency followed by a decrease, and about one week after inoculation the efficiency drops

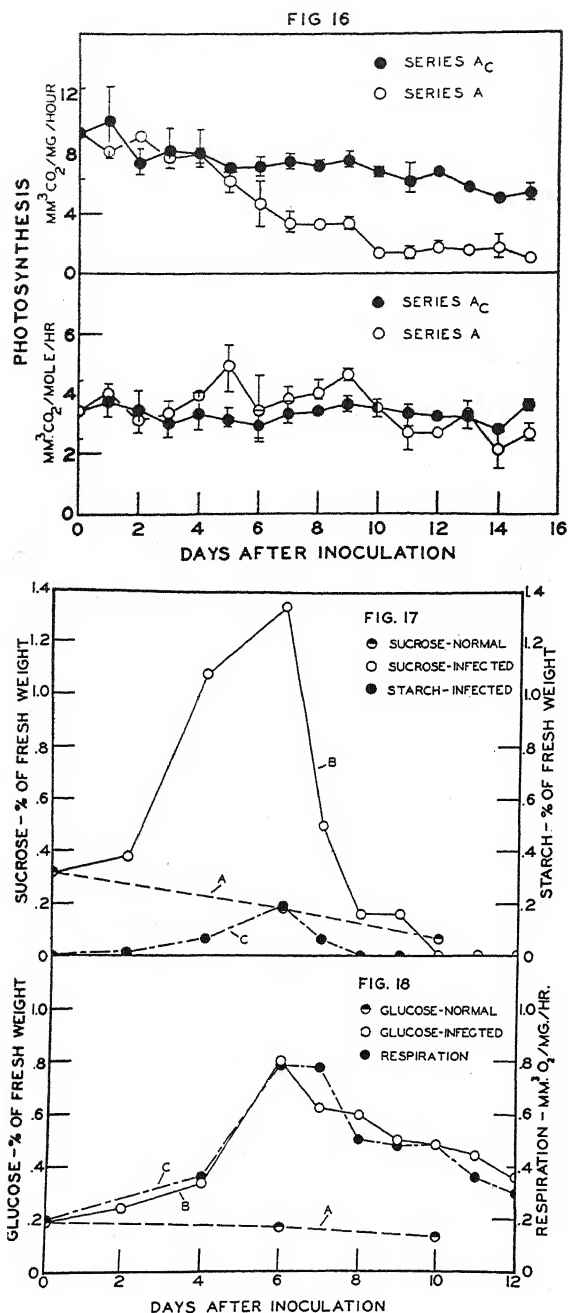


Fig. 16-18.—Fig. 16. Photosynthesis at incomplete light saturation (150 foot candles) of Marquis wheat leaves grown in the greenhouse in August. Note the greater uniformity in the curves for normal leaves (series A_C) when light intensity is the limiting factor.—Fig. 17-18. Changes in carbohydrates in leaves of Marquis seedlings grown in control chamber, and the relation between glucose content and respiration. Figures 17A and 18A represent the changes in sucrose and glucose, respectively, in normal wheat. The other curves represent changes in heavily infected leaves of series F. All plants were grown in the control chamber. Note the close parallelism between glucose content and respiration.

below its original level. In the lightly infected leaves of series E it does not increase, nor does it drop appreciably below normal until late in the course of the infection. A definite drop appears on the same day that the final decline in respiration starts. This figure, like the others for lightly infected leaves, shows considerable variability in the samples taken on any given day. The difficulty in obtaining uniform inoculation makes it impossible to carry the interpretation of these curves very far.

The absence of any relation between photosynthetic rate and chlorophyll content is in agreement with the recent work on photosynthesis (Ruben, Kamen, and Hassid, 1940) which indicates that CO_2 is fixed by some compound other than chlorophyll, and that this compound may be varied independently of the chlorophyll content.

Photosynthesis in low light.—The changes in photosynthesis at 180 foot candles, where the normal rate is somewhat less than half maximum, were determined for series A_C and A (Marquis seedlings grown in the greenhouse in summer). The resulting curves are shown in figure 16. Here the photosynthesis of normal leaves declines slowly over a two-week period, but does not show nearly as many fluctuations as under a saturating intensity of light. Calculation of the photosynthetic efficiency of these leaves yields an even more uniform curve in which many of the fluctuations which appeared in high light are absent. These curves conform with the expected trend of photosynthesis in normal leaves when light intensity is a limiting factor. In low light the dark reactions proceed more rapidly than is necessary to take care of the products of the light reactions, and variations in their activity are, therefore, not reflected by corresponding changes in the rate of photosynthesis. Variations in the chlorophyll content are, however, effective in altering the observed rate of photosynthesis, as the constancy of the values for photosynthetic efficiency shows.

In diseased leaves, the curve for photosynthesis does not show any clear cut difference from that obtained in high light. Calculation of the photosynthetic efficiency, however, yields a curve which does not drop below the normal until eleven days after infection, instead of seven days afterwards as in high light. Here again photosynthesis is more dependent on chlorophyll content than it is in high light, and not until the dark reactions have been slowed down considerably more than in high light does their activity exert a rate-determining effect on the overall process of photosynthesis.

Glucose and sucrose.—The sugar content of normal leaves grown in the control chamber declines gradually after the leaves are mature (fig. 17A and 18A). Upon inoculation with mildew there is an accumulation of both glucose and sucrose (fig. 17B and 18B). In the plants of series F sucrose has increased, four days after inoculation, from .32 per cent of the total fresh weight to 1.08 per cent, but there is as yet no appreciable increase in glucose. Subsequently sucrose continues to accumulate, and glucose also

increases greatly. The disappearance of soluble sugars, like their appearance, is brought about first by a decrease in sucrose; while the glucose tends to maintain a more uniform concentration. This behavior of the soluble sugars is similar to that observed by Hassid and McCready (1941), who have recently demonstrated that changes in the sugar content of barley leaves appear primarily as changes in the amount of sucrose, while glucose tends to remain at a fixed level.

The changes in respiration in series F are shown in figure 18C. Respiration, glucose, and sucrose are all at a maximum on the same day, six days after inoculation. The changes in respiration show a good correlation with changes in glucose content, but no relation to sucrose. The correlation is particularly close up to the respiratory maximum. After this point a close correlation is not to be expected, because, as was pointed out above, in heavily infected leaves carbohydrate is not the only respiratory substrate during the phase of declining respiration. These experiments point to the glucose content as a primary factor in determining the respiratory rate. There is no correlation between total sugars and respiration, such as Krotkov (1939) found in starving leaves of Little Club wheat.

Starch.—The normal wheat leaf does not form starch except in the guard cells, where the chloroplasts appear as dark bluish-black granules when stained with I-KI. But as soon as a mildew colony becomes established on the leaf, a dark discoloration appears immediately underneath and extending slightly beyond the mycelium. By the time that the fungus is sporulating, five or six days after inoculation, the chloroplasts of cells near a mildew colony show the same dark staining material that appears in the guard cells. Its color and its association with the chloroplast indicate that it is starch, although other substances as well contribute to the dark discoloration in diseased leaves. At about the time when respiration is highest, starch is most abundant, and subsequently it disappears. But the dark discoloration around the mildew colonies persists even after the disappearance of recognizable granules containing starch. Dr. Pratt⁴ has observed similar areas of discoloration when diseased leaves are stained with other reagents, such as methylene blue. The localized spread of the mildew toxins could not be better demonstrated than by these areas of discoloration around each colony (fig. 19). Not only is the spread localized, but it is more restricted laterally than it is longitudinally. Lengthwise of the leaf, transport is facilitated by the elongated cells of the epidermis and vascular tissues, so that the shape of the discolored areas is elongate ellipsoidal. The close correspondence between the form of the colonies and the form of the areas which show by the deposition of starch that they have been affected by the mildew toxins suggests very strongly that the changes produced by the mildew are necessary for the rapid development of the parasite.

⁴ Private communication.

Quantitative analyses for starch were made on the leaves of series F after the sugars had been removed. The amount of starch in normal leaves is too small to measure, while in the diseased leaves it accumulates to a maximum value of about .2 per cent (fig. 17C). Like the iodine tests these analyses show a maximum accumulation of starch at the time of the

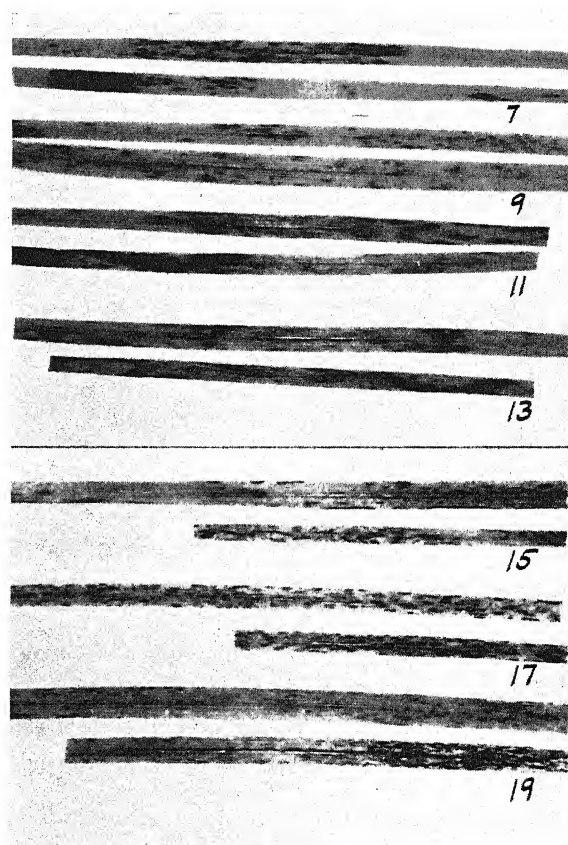


Fig. 19. Leaves at various stages after inoculation with mildew, showing the distribution of areas stained with I-KI. The age of the infection is indicated by the figures on the right. Each dark spot represents the effect of a mildew colony. Note the longitudinal spread of the discoloration under young colonies (seven days). In older leaves most of the starch is gone (fifteen to nineteen days) except where secondary infection has developed (nineteen days).

most rapid respiration and the greatest accumulation of soluble sugars.

An abnormally large amount of starch and sucrose is found in mildewed wheat plants only during the first eight days after infection. Thereafter both starch and sucrose disappear completely. Observations made at an early stage of the disease would, therefore, lead to the conclusion that starch and sucrose had increased as a result of infection, while if they were made later they would lead to the conclusion that sucrose had been destroyed. This may account in part for the divergent results of previous

investigators, who have sometimes found that parasitic infection caused an accumulation of carbohydrate in the host (Schellenberg, 1911) and at other times have found a decrease (Robinson, 1913, and others).

Formation of green islands.—Green islands are localized areas containing chlorophyll in leaves which have elsewhere become chlorotic as a result of parasitic infection. They were first reported by

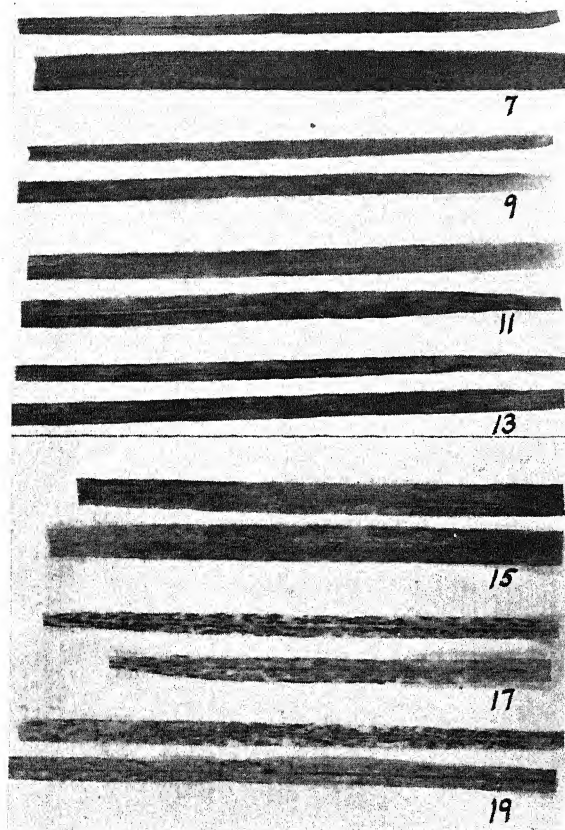


Fig. 20. The development of green islands on infected leaves. Photographs taken from the uninfected side of the leaf. Under the young colonies chlorotic spots appear (seven, nine days); later green spots appear in the center of the chlorotic areas (eleven to fifteen days), and eventually are left as green islands when the areas of chlorosis fuse (seventeen, nineteen days).

Cornu (1881) on leaves of several deciduous plants infected with both obligate and facultative parasites, and have since attracted the attention of many investigators. They are particularly striking in leaves infected with mildew when the carbohydrate reserves of the host are low. For instance, if diseased leaves are left in the dark for a couple of days, the leaves quickly become chlorotic except underneath the mildew colonies; or, if photosynthesis is particularly low in the greenhouse, as it is during winter or in heavily shaded leaves, green islands appear.

In the normal course of development on vigorous leaves a mildew colony first causes a destruction of

chlorophyll in the cells immediately beneath the mycelium (fig. 20, seven and nine days). As the colony enlarges, the chlorotic area also enlarges, spreading somewhat faster than the fungus. At the time when starch begins to disappear, as indicated by the iodine staining (which is also the time of disappearance of soluble sugars), a spot of green appears in the center of each chlorotic area. The green spot enlarges, leaving a ring of yellow surrounding it (fig. 20, thirteen and fifteen days). With the continued spread of the chlorotic areas, adjacent regions of chlorosis fuse, eventually occupying the entire leaf except for the green islands underneath the mildew colonies (fig. 20, seventeen days). Green islands do not, therefore, represent merely the retention of chlorophyll in cells affected by the mildew, but they actually are areas in which the chlorophyll was first destroyed during the phase of rapidly changing host metabolism, and was subsequently re-formed. Reference to figures 9, 10, and 11 shows that the total chlorophyll content stops decreasing when it is still at a fairly high level, and, in the case of series D, figure 10, there is definitely an increase above the low level reached ten days after infection. The fact that, at the same time, photosynthesis continues to decrease (fig. 6–8) indicates that the chlorophyll which appears in the green islands is not photosynthetically active.

Discussion.—The changes in host metabolism brought about by mildew infection divide themselves conveniently into two phases, which we shall refer to as the first and second phases of the disease. The characteristics of host metabolism in these two phases are fundamentally different and appear to be separated by a rather sudden disorganization of the normal metabolic relationships at the time of the maximum in respiration and carbohydrate accumulation. Up to that time the changes which occur are primarily quantitative; subsequently marked qualitative changes also occur. During the first phase the host affords an excellent substratum for mildew growth and the vegetative expansion of the mildew is rapid; subsequently little vegetative growth occurs, and the ability of the mildew to sporulate drops off. These two phases are most distinct in heavily infected leaves, where the entire leaf soon comes under the influence of the mildew toxins, and the picture is not complicated by secondary infection.

The first phase is characterized by an increase in the rate of oxidation of carbohydrate; by an increase in glucose, sucrose, and starch; by a constant glucose respiration ratio; and by a destruction of chlorophyll which is closely followed by a decrease in photosynthesis but not by a decrease in photosynthetic efficiency. The time during which these changes proceed seems to correspond with the time required for the mildew toxins to spread widely throughout the leaf, because the first phase lasts considerably longer in lightly infected leaves, and its features predominate as long as new leaf areas are coming under the influence of growing mildew

colonies. The fact that the increase in respiration and the decrease in photosynthesis proceed independently of each other during this phase even though they both reduce the normal supply of carbohydrate, indicates that this is a period during which a third agent, presumably the mildew toxins, is a major factor determining the trend of these processes. Only after photosynthesis has been reduced to a very low level does the effect of carbohydrate starvation enter as a factor modifying the effect of the mildew toxins on the carbohydrate breakdown and the carbohydrate content of the leaves.

The second phase is opened by a rather abrupt change in all of the above mentioned relationships. The rate of cellular oxidations drops off rapidly, and a decrease in R.Q. indicates that now other substrates besides carbohydrate are being oxidized. This is corroborated by the fact that respiration is no longer strictly proportional to glucose concentration (fig. 18B and C). All of the three types of carbohydrate investigated decrease in quantity, sucrose and starch disappearing completely. The rate of photosynthesis continues to drop, but now more rapidly than chlorophyll, so that the photosynthetic efficiency falls below normal. During the early part of this phase the cells first affected by the mildew form new chlorophyll, which is, however, inactive in photosynthesis. Vegetative growth of the mildew slows down, and soon afterwards undisturbed colonies present the aspect of tiny craters, because the failure of the central part of the colony to continue forming conidia results in a small central depression surrounded by the chains of conidia on the younger hyphae near the periphery. It is during this second phase of the disease that green islands first appear.

The experiments of Trelease and Trelease (1929) demonstrated very clearly the dependence of mildew on an adequate supply of carbohydrate to the host. The above experiments, which were carried out using the same strain of mildew used by the Treleases, show that, in the development of mildew on intact plants, the fungus does not grow merely on the carbohydrates normally present in the wheat leaf but that, by preventing their removal from the leaf, it plays an active role in securing for itself an abundance of carbohydrates. The advantage which this gives to the mildew may result directly from the utilization of carbohydrate by the fungus. Since, however, the host is at the same time respiring carbohydrate rapidly, it is equally possible that other substances resulting from the metabolism of these sugars

are of vital importance in the vegetative development of the mildew. In any case, the high carbohydrate content and the high respiration are intimately connected with mildew growth. This is clear from the close correlation between the periods of mildew growth and the periods of carbohydrate accumulation and increasing respiration. In winter grown plants the phase of increasing respiration (and, therefore, presumably of increasing sugar content) does not last very long and the rate does not increase greatly. On such leaves the mildew colonies develop slowly and never become very large. On heavily infected leaves of vigorous seedlings the colonies develop more rapidly and become larger, while on lightly infected leaves, in which the first phase lasts much longer, the colonies reach the largest size of all.

The effects of the mildew are not, however, all to its advantage. The increased respiration resulting from the accumulation of carbohydrate together with a decreasing rate of photosynthesis eventually leads to the depletion of the host carbohydrate reserves. At the same time, either as a result of, or as a corollary to the increased respiration, other components essential to the life of the cell are destroyed. If the leaves are heavily infected, they may die before the mildew has a chance to form resistant ascospores, and its only method of reproduction is by the abundant but non-resistant conidia.

In the normal wheat leaf the rate of carbohydrate synthesis far exceeds the rate at which it is respired. Very little is stored in the leaf as soluble sugar and none as starch, so that the majority of it must be exported to other parts of the plant which do not form their own carbohydrate, such as the root and the growing parts of the shoot. The accumulation of sugars and starch and the more rapid respiration in diseased leaves must depend on some influence of the parasite which prevents the normal export of soluble sugars. In fact, since the rate of photosynthesis is dropping rapidly while an increase in the accumulation and breakdown of carbohydrates is going on, there is even some question as to whether the photosynthesis of the diseased leaf is sufficient to supply the demand. In order to give some idea of the relative amounts of carbohydrate produced and those used up at different times after inoculation a balance sheet has been prepared and is shown in table 2. All except the values for photosynthesis are taken from series F. Since photosynthesis was not measured in that series, the figures from series D, which

TABLE 1. Summary of the culture conditions in different series.

Series	Fe source	Growth conditions	Seed	Age at inoculation
A and A _e	Fe tartrate	Greenhouse in August	Marquis	10 days
B	Fe tartrate	Greenhouse in December	Marquis	10 days
D	Fe citrate	Control chamber	Axminster	12 days
E	Fe citrate	Control chamber	Marquis, pure line	12 days
F	Fe citrate	Control chamber	Marquis, pure line	12 days

TABLE 2. *The figures in this table represent moles of carbon per unit weight of leaf per day. The first two rows give the amounts of carbon synthesized and respired on successive days after inoculation. The third is the excess of carbon synthesized over that respired. In the fourth row is given the amount of carbon found in the form of carbohydrate. Subtracting this from the amount not respired gives the carbohydrate available for export on any given day, shown in the last row of figures.*

Age of infection:	0	2	4	6	7	8	9	10	11	12
Photosynthesis	45.3	43.0	30.2	19.9	14.6	6.7	6.9	4.4	3.8	3.0
Respiration	2.1	3.0	3.4	8.6	8.0	5.4	5.2	5.3	4.0	2.9
Balance	43.2	40.0	26.8	11.3	6.6	1.3	1.7	-0.9	-0.2	-0.1
Carbohydrate	1.7	2.0	5.0	7.8	3.7	2.5	2.2	1.6	1.5	1.2
Carbohydrate available for export..	41.5	38.0	21.8	3.5	2.8	-1.2	-0.5	-2.5	-1.7	-1.3

has a similar respiratory curve, were used as a basis for estimation. In the control chamber CO_2 concentration was approximately atmospheric and probably, therefore, a limiting factor in photosynthesis. To allow for this the values of series D were reduced to two-thirds. Light intensity in the chamber was about 200 foot candles, which would give a photosynthetic rate one-half maximum. The figures for photosynthesis are, therefore, obtained by dividing the maximum values by one-third. The values obtained in this way show that by the time respiration and sugar content are at a maximum there is little or no surplus carbohydrate available for export. No statement concerning an actual import can be based on these figures. The fact that sugars and starch begin to disappear as soon as photosynthesis becomes too low to provide a surplus argues against a flow of sugars into the diseased leaves.

The accumulation of starch might be the result of the increased concentration of sugar. But the fact that starch is never found in normal wheat leaves (except the guard cells), while the sugar content of such leaves under unusual conditions may be as great as 2.5 per cent, indicates that other changes must take place before starch is formed. Such a change might be produced by an increase in phosphorylase, which Hanes (1940) has recently shown to be the enzyme responsible for starch synthesis in higher plants.

SUMMARY

When wheat leaves are lightly infected with mildew, the respiration curve exhibits two maxima, one corresponding to the time of maturity of the primary colonies, the other to the time of maturity of the secondary colonies.

The respiratory maximum in plants grown in the greenhouse in winter is only about one-third as great as that of plants grown under better lighting conditions.

The growth of a mildew colony is rapid during the period of increasing respiration, sugar, and starch. After these reach their peak and start declining, vegetative growth of the mildew slows down. The individual colonies on heavily infected plants, where respiration is at a maximum a week after inoculation,

are, therefore, smaller than those on lightly infected ones, where the respiration is not at its height for about two weeks.

The substrate oxidized in lightly infected leaves, as indicated by the R.Q., is mainly carbohydrate; in heavily infected leaves carbohydrate is the primary substrate up to the respiratory maximum, but thereafter other substances are also oxidized.

Mildew infection leads to a breakdown of the photosynthetic mechanism which involves both the light and the dark reactions. In heavily infected plants, chlorophyll content and photosynthesis at light saturation (600 foot candles) start dropping rapidly soon after inoculation. They do not, however, follow the same course, since the photosynthesis per mole of chlorophyll first increases, then drops off, falling below normal about a week after inoculation. In lightly infected plants at light saturation the decreases in photosynthesis and chlorophyll are more gradual, and the photosynthesis per mole of chlorophyll does not increase. About two weeks after inoculation, when respiration is declining from the second maximum, it drops abruptly. In heavily infected plants at incomplete light saturation the changes in photosynthesis are more closely related to chlorophyll content.

In heavily infected plants there is an accumulation of soluble sugars which reaches a maximum simultaneously with respiration. Sucrose accumulates more rapidly than glucose, reaches a higher level, and disappears again more rapidly.

The rate of respiration up to the maximum is proportional to the glucose content but is not related to sucrose. After the maximum, respiration is nearly proportional to glucose content but not so closely as before.

In infected wheat leaves, starch is deposited near the mildew colonies. It is most abundant at the time of the maximum in respiration and soluble sugars, and disappears as they decrease.

During the early growth of the mildew, chlorotic spots appear beneath each colony and spread outward until they fuse. Before the fusion is complete, at about the time of the respiratory maximum, chlorophyll is re-formed under the center of each mildew colony and spreads outward to form green islands,

each of which occupies an area about the size of a mildew colony.

An estimation of the excess of carbohydrate produced over the amount utilized by the leaf shows that the amount available for export decreases to zero or below at the time of the respiratory maxi-

mum. Thereafter all the carbohydrate synthesized is utilized by the leaf itself.

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STUDIES ON THE BIOLOGY OF TWO SPECIES OF MAGNUSIA. II. EFFECT OF HUMIDITY ON CONIDIAL GERMINATION, GROWTH AND REPRODUCTION¹

Herman R. Sweet

IN THE previous paper dealing with the effects of temperature on spore germination, growth and reproduction, the writer (1941) pointed out that desiccation had a marked influence both on the germination of the spores and on the growth of *Magnusia nitida* Sacc. and *M. brachytricha* Ames. As a result, experiments were made to discover the degree of humidity necessary for the activity of these two fungi and to determine the limits at which germination, growth and reproduction would take place.

EFFECT OF HUMIDITY ON CONIDIAL GERMINATION.—For this study, potato-rat dung agar as described in the previous paper was used as a substratum, the agar being placed on cover glasses which were then inoculated with the conidia, inverted, and sealed to Van Tieghem cells containing various percentages of sulphuric acid, according to the procedure outlined by Stevens (1916). Three slides of each species for each relative humidity, placed in a constant temperature oven at 32°C., were examined at hourly intervals up to twelve hours, and then at twenty-four, forty-eight, and one hundred hours. The number of conidia per slide averaged 200, the two extremes being 150 and 250. The relative humidities used in the first series were as follows: 100, 91.2, 80.6, 70.4, 60.7, 49.0, and 29.5 per cent; and in the two additional series were 100, 99.5, 99.1, 98.7, 98.2, 97.5, 95.6, and 93.9 per cent.

The results of the first series of experiments, in which the wider range of relative humidities was employed, demonstrated that the conidia of both *M. nitida* and *M. brachytricha* germinated at or near 100 per cent relative humidity, with only one slide germinating at the next lower humidity of 91.2 per cent where at the end of fifty hours no conidia had germinated, but at the end of one hundred hours the spores of *M. brachytricha* on one slide showed a germination of 23.8 per cent. The range for conidial germination was, therefore, somewhere above a relative humidity of 91.2 per cent. When examined under the microscope, it was seen that the conidia kept at humidities under 91.2 per cent had lost water, as was shown by their reduced size, and conidia maintained in an atmosphere of 70.4 per cent relative humidity or lower were definitely collapsed and smaller in size than those above 70.4 per cent relative humidity.

The results of the two additional series of experiments are shown in figure 1. As is obvious from the

graph, the conidia of *M. nitida* germinated both more readily and at a lower humidity than did those of *M. brachytricha*. At a relative humidity of 99.5 per cent *M. nitida* germinated to the extent of 96.7 per cent in one of the series, and *M. brachytricha* to the extent of 91.8 per cent, whereas there was no germination of *M. nitida* in a relative humidity of less than 95.6 per cent and of *M. brachytricha* in a humidity of less than 97.5 per cent. At both 100 and 99.5 per cent relative humidities, germination had begun to take place by the end of the fourth and fifth hours, respectively, but below 99.5 per cent the time required for the initiation of germination was lengthened, the first conidia of *M. nitida* germinating at the end of six hours and those of *M. brachytricha* some time between eight and twenty-four hours. A greater percentage of germination was obtained at a rela-

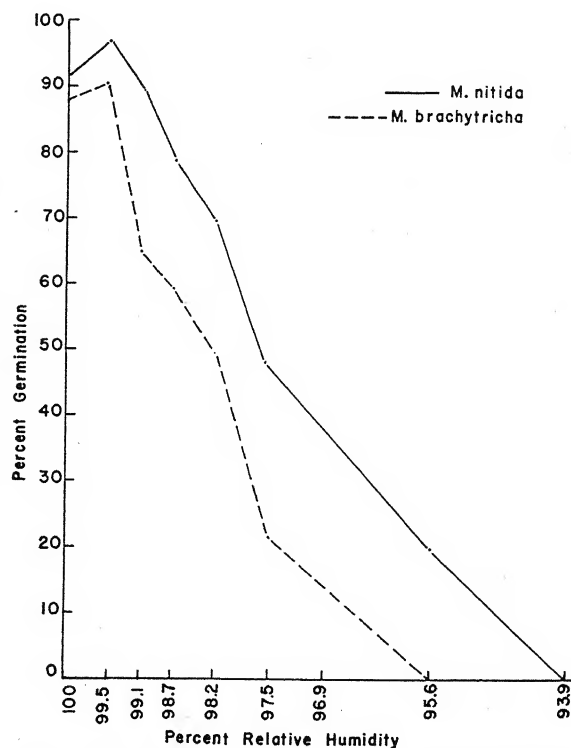


Fig. 1. The effect of various humidities on germination of conidia of *M. nitida* and *M. brachytricha*.

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tive humidity of 99.5 than at 100 per cent. At first thought, it would seem that a difference in humidity of 0.5 per cent would be too small to make any significant difference in the results, but Longrée (1939), in a careful series of experiments using conidia of *Sphaerotheca pannosa* var. *rosae*, also demonstrated that in humidities just under 100 per cent—that is,

at 99.0 and 98.0 per cent—there was a greater percentage of germination than at 100 per cent. In her experiments, at a relative humidity of 96.9 per cent, the spores germinated poorly, whereas at 94.9 per cent only a few (2.0 per cent) germinated. The lower percentage of germination at 100 per cent relative humidity was found to be due to free water deposited on the spores, and later experiments showed that the free water (water of condensation) caused a deficiency of oxygen. It is perfectly possible that this is the explanation for the lower percentage of germination at 100 per cent relative humidity in these species of *Magnusia*, since it was noted in the course of other experiments that a higher percentage of germination was obtained on hanging drops of agar that had little free water than on agar drops suspended in a saturated atmosphere. It was also found that conidia resting on the surface of agar drops, where the humidity is slightly lower and the supply of available oxygen greater, germinated more readily than did those below the surface of a water droplet.

The fact that a high relative humidity is necessary for the germination of many species of fungi has been pointed out by several workers. Linder (1929) found that there was practically no germination of helicosporous fungi at a humidity lower than 98.8 per cent. Melhus, Durrell and Kirby (1920) found that the teleutospores of *Puccinia graminis* did not germinate at a relative humidity below 95.6 per cent, and Maneval (1922) likewise found that a saturated atmosphere was necessary for the germination of the teleutospores of *Puccinia Helianthus* Schw.

Because in nature the fungi must be subjected to drying and wetting, it was considered advisable to test the resistance of the conidia to desiccation under conditions existing in the preliminary experiment as contrasted with drying in the room. To this end, the conidia that had been subjected in the early experiments to relative humidities of 91.2 per cent and below were transferred at the end of one hundred hours to other Van Tieghem cells in which the sulphuric acid solutions had been replaced by distilled water, thereby establishing a saturated atmosphere. The conidia then absorbed water to such an extent that at the end of twenty-four hours they had returned to approximately their normal size and some had germinated. Those which failed to germinate also failed to show the characteristic swelling which occurs prior to germination.

This experiment reaffirmed the greater ability of *M. nitida* to recover from adverse conditions, for this species showed not only a higher percentage of germination than did *M. brachytricha*, but also germination occurred on slides kept previously at the lower humidities. Of the slides formerly kept at 91.2 per cent relative humidity, the conidia of *M. nitida* alone made a good recovery, achieving a germination of 75.8 per cent at the end of twenty-four hours, and 95.0 per cent at the end of forty-eight hours, whereas those of *M. brachytricha* showed a 34.6 per cent germination at the end of twenty-four hours,

and only 50.0 per cent in twice that time. Of the conidia which had been kept at a relative humidity of 70.4 per cent for one hundred hours, those of *M. nitida* showed a 12.6 per cent germination at the end of twenty-four hours, and a 40.0 per cent germination at the end of forty-eight hours in a saturated atmosphere, but those of *M. brachytricha* showed only a 25.0 per cent germination at the end of forty-eight hours. Of the conidia previously kept at a relative humidity of 60.7, there was no germination of *M. brachytricha*, but there was a 10.0 per cent germination of *M. nitida* at the end of forty-eight hours. In all cases, there was a considerable delay in the initiation of germination, and the time required for the conidia to reach their maximum germination was greatly lengthened.

To compare the effect of drying at higher temperatures with that at room temperature, conidia from cultures approximately two weeks old were placed on glass slides in Petri dishes, each of which was kept at a specified temperature for a definite period of time. *M. nitida* was again revealed as able to withstand adverse conditions somewhat better than *M. brachytricha*, since conidia kept at 24°C. for forty-eight hours germinated to the extent of 94.6 and 93.0 per cent for *M. nitida* and *M. brachytricha* respectively, while, when the conidia were kept at 24°C. for ninety-six hours and then at 38°C. for twenty-four hours, the germination amounted to 70.2 and 47.0 per cent for the two respective species. However, the difference between the two species was not so great when the conidia were dried for ninety-six hours at 24°C. and for sixty hours at 38°C., since the percentage of spore germination of *M. nitida* was then 43.0, whereas that of *M. brachytricha* was 41.2. It is apparent that the harmful effects of desiccation became manifest more quickly at the higher temperatures, since, when the conidia were kept protected in a Petri dish at room temperature for a year and a half, during which time the temperature varied from 20 to 24°C., 45.0 per cent of the conidia of *M. brachytricha* and 48.5 per cent of *M. nitida* germinated. When these results are compared with those of the previous experiments, it would seem that a year and a half of desiccation at room temperature had the same effect as sixty hours at 38°C.

Low humidity has been noted by other investigators as having an almost universal influence upon the viability of spores. Linder (1929), for example, found that *Helicoma Mülleri*, *Helicosporium aureum* and *H. gracile* did not germinate after being kept for nine months at the humidity and temperature of the laboratory. He did find two of the species germinating slightly after a similar exposure of eleven months: *Helicomycetes scandens* and *Helicoma Curtissi*, the latter to an extent of 4.8 per cent. Newhall (1938) found the conidia of *Peronospora destructor* able to survive several days at humidities above 70.0 per cent at room temperature. Sawyer (1931), however, found that *Entomophthora sphaerosperma* did not remain viable unless placed at a low temperature.

It would be inaccurate to conclude from these experiments that in nature such a high degree of atmospheric humidity is essential to the germination of the conidia, since the porosity and moisture of the substratum would play an important part in germination, for a porous substratum and an abundance of capillary water would allow the fungi to germinate at humidities apparently below those at which germination occurred under laboratory conditions. A hanging droplet of nutrient agar would naturally

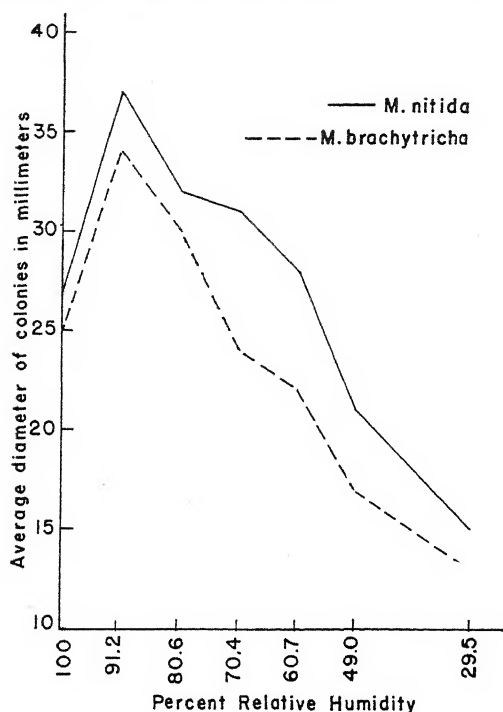


Fig. 2. The effects of various humidities on mycelial growth of *M. nitida* and *M. brachytricha*.

dry out more rapidly than the natural substratum because the sulphuric acid in the bottom of the Van Tieghem cells absorbs all excess humidity above a given percentage. The natural substrate might well have contact with damp earth and be kept moist by capillary water. Zeller (1920) points out that the saturation point of wood fiber is reached at 95.0 per cent relative humidity, below which point the spore germination of basidiomycetous wood-destroying species is low, for the fibers seem to demand water at their expense. Above this point, germination is as high as 75.0 per cent and higher. Probably a similar condition would exist in nature in the case of these species of *Magnusia*.

EFFECT OF HUMIDITY ON GROWTH AND REPRODUCTION.—To discover the effects of humidity on growth and reproduction, a series of experiments was carried out somewhat similar to the first series on the effect of humidity on conidial germination.

To obtain the desired relative humidity for Petri dish cultures, desiccators 9 inches in diameter and

7¼ inches high were used. Seventy-five mm. Petri dishes containing potato-rat dung agar were inoculated with square blocks of agar of uniform size containing the mycelium of *M. nitida* and *M. brachytricha*. Two plates of each species were made up for each humidity in each series. The plates were inverted, covers removed, and the cultures were placed on the perforated porcelain plate above the sulphuric acid solution. The covers of the desiccators were then sealed, and the whole apparatus was kept at room temperature (from 20°C. to 24°C.) in indirect light. The following relative humidities were used: 100, 91.2, 80.6, 70.4, 60.7, 49.0, and 29.5 per cent.

As was found in the experiment on the effect of humidity on conidial germination, the optimum relative humidity for growth was below 100 per cent; in the case of mycelial growth, it was at 91.2 per cent, at which point *M. nitida* grew an average of 37 mm. and *M. brachytricha* an average of 34 mm. in nineteen days (fig. 2). Below 91.2 per cent relative humidity there was a gradual decrease in the diameter of the colonies of both species, which was particularly noticeable at 60.7 per cent and below. At every relative humidity *M. nitida* grew more extensively than did *M. brachytricha*.

The amount of aerial mycelium (table 1) produced by each species varied with the humidity. At 100 per cent relative humidity the aerial mycelium of both species was long, fluffy, and loose in texture, but at 91.2 per cent the aerial mycelium of *M. nitida* was much shorter and more compact, whereas *M. brachytricha* produced none. At 80.6, 70.4, and 60.7 per cent there was a decrease in the amount produced by both species—a decrease noted chiefly in the length of the hyphae making up the aerial mycelium. At 49.0 per cent relative humidity and below, no aerial mycelium was produced by either species and, while growth occurred, it was obvious that this had taken place before an equilibrium between agar and atmosphere had been reached. It is doubtful if further growth after inoculation would have occurred had there been an immediate equilibrium between the substratum and the atmosphere. In general, *M. nitida* produced aerial mycelium more consistently and over a wider range of humidity than did *M. brachytricha*.

The production of conidia in both *M. nitida* and *M. brachytricha* (table 1) was excellent down to 60.7 per cent relative humidity, but at 91.2 per cent the conidiophores were more numerous than at either 100 or at 80.6 per cent or lower. In one series of experiments the conidia were consistently produced uniformly over the surface of the colonies of both species, and in the other series, conidia were just as consistently formed only in the center of the colonies of both species. Since in both series the same lot of medium was used, external factors were apparently responsible for this slight variation in conidium production.

The range of humidity for cleistothecium production in *M. brachytricha* was from 100 down to 49.0

TABLE 1. Effect of controlled relative humidity on growth and reproduction at the end of nineteen days at 24°C.

Per cent relative humidity	Av. diam. in mm.	<i>Magnusia nitida</i>				<i>Magnusia brachytricha</i>			
		Aerial mycelium	Conidia	Cleisto-thecia	Condition of agar	Av. diam. in mm.	Aerial mycelium	Conidia	Cleisto-thecia
100.0	27.0	Long, loose, sparse	5 ^c	4 ^a	Moist	25.0	Long, loose, sparse	4 ^c	4 ^a
91.2	37.0	Fairly loose; sparse; sterile white	5	5	Moist	34.0	None	5	5
80.6	32.0	Short; less abundant	4	4	Fairly moist	30.0	Slight amount	5	4
70.4	31.0	Less apparent	4	3	Fairly moist	24.0	Slight amount	4	3
60.7	28.0	Slight amount	3	2	Dry	22.0	Slight amount	4	2
49.0	21.0 ^a	None	3	1	Dry	17.0	None	3	0 ^b
29.5	15.0 ^a	None	1	1	Very dry	13.0 ^a	None	1	0

^a In a check experiment using less agar, agar is completely dried out and no growth is visible.^b In a check experiment, a few immature cleistothecia.^c Relative number of conidiophores.^d Relative number of cleistothecia.

per cent relative humidity, although, at this latter humidity, in only one series were a few immature cleistothecia formed. The range in *M. nitida* was from 100 to 29.5 per cent, at which latter point a few cleistothecia were formed in one series of cultures. In view of the previous experiments in which growth at low humidity did not take place, it seemed desirable to determine whether or not variations in the amount or depth of agar in the Petri dishes would explain this variation in behavior. Accordingly, in a subsequent series, in which less agar per Petri dish was used, it was found that the agar dried out at both 49.0 and 29.5 per cent relative humidities and as a result no cleistothecia were formed, nor was there any visible growth. Since in neither series did *M. brachytricha* produce any cleistothecia at 29.5 per cent relative humidity, the range for the production of cleistothecia in *M. nitida* would seem to be a little wider than that in *M. brachytricha*. For both species, the optimum of the relative humidities used was 91.2 per cent, which, it will be recalled, is also the optimum for growth and for conidium formation.

Although a humidity of 91.2 per cent seemed to be the optimum for both conidium and cleistothecium production in the two species of *Magnusia*, such a high humidity has not been universally found necessary for the optimum development of the reproductive bodies. Robinson (1926), for example, found that the sexual reproduction of *Pyronema confluens* was best at humidities ranging from 68.5 to 52.0 per cent, but Reidemeister, cited in Hall (1933), found that high humidity favored conidium formation in *Botrytis cinerea*, and Coons (1916) found that moisture had an inhibiting effect upon pycnidium formation of *Plenodomus fuscomaculans*.

At humidities below 91.2 per cent the amount of agar medium per Petri dish undoubtedly plays an important part in the percentage of cleistothecium formation obtained, for the greater the amount of agar, the greater is the amount of moisture from the agar available to the fungi. In effect, there are two factors to be considered in the humidity experiments: the moisture content of the air and the availability of moisture in the substrate. It is possible to control in some measure only the former. At the lower humidities there is, of course, a constant loss of water from the substrate. As already pointed out, if moisture is available in the substrate, cleistothecia will be produced at lower humidities than if it is not available. Below a certain critical point there seems not to be sufficient moisture in the medium to make up for the lack of moisture in the air.

Other investigators are agreed that growth and reproduction occur best at fairly high relative humidities. Fisher (1939), for example, found that the sooty moulds grew best at 100 and 99 per cent relative humidity, with the lower limits of growth at 92.5 per cent. McCrea (1931) found that abundant moisture was needed for the growth of *Claviceps purpurea*. Walter, cited by Tomkins (1929), found that *Penicillium* grew independently of the relative humidity down to 95 per cent, and that *Aspergillus*

glauca grew at a lower relative humidity than *Penicillium glaucum*, although there was no growth below a relative humidity of 85.0. Although the same criticism can be made of the *Magnusia* experiments that apply to Walter's (1924) work, namely, that the temperature of the experiments was not constant, and that moisture present in the drop had to come into equilibrium with the air, it should be kept in mind that in nature a perfectly constant temperature is not to be expected, and an interchange of moisture between the substrate and the surrounding atmosphere would be of normal occurrence. Under laboratory conditions, in the enclosure of the desiccator, a relatively constant equilibrium would be reached which would not obtain in nature, where a fairly free circulation of air or moisture drawn from soil may make the equilibrium reversible.

During the course of these investigations, attention was paid to the occurrence of sector mutants in cultures subjected to varied environmental factors, in the hope that some light might be thrown on factors causing this phenomenon. In several of the plates at 70.4 per cent relative humidity and above, sector mutants occurred, most of which were composed of sterile white mycelium. The one exception was *M. brachytricha* at a relative humidity of 80.6, which had a dark sector composed of black mycelium growing in the agar; none of the mycelium was aerial. The sector was isolated and has remained true in culture to the original black type for over three years. Since sector mutants arose in all colonies in which there was rapid vegetative growth, it would seem that they did not arise as a result of unfavorable environmental conditions but that the cause for their appearance must be sought either in the nature of the medium or in the behavior of the nuclei in a thallus that has resulted from rapid vegetative growth with the accompanying repeated nuclear divisions. As a result of aberrant nuclear division it is possible that genetic characters were not divided equally between the daughter nuclei so that when the nuclei with atypical genetic structure became segregated in a branch of the mycelium, this branch gave rise to the so-called mutant.

SUMMARY

The effect of humidity on conidial germination, on growth and reproduction of the two species of *Magnusia* may be summarized as follows:

The range of humidity in which conidial germination took place was somewhat wider for *M. nitida* than for *M. brachytricha*—from 100 through 95.6 per cent relative humidity for *M. nitida*, and from 100 through 97.5 per cent for *M. brachytricha*, with an optimum for both species at 99.5 per cent relative humidity.

The conidia of *M. nitida* made a better recovery from drying than did those of *M. brachytricha*.

There was no difference in the range for growth of the two species, since both were able to persist in the full range of relative humidities from 29.5 to 100 per cent, with an optimum for both species at about 91.2 per cent relative humidity.

The range for the production of conidia in both species was from 100 to 60.7 per cent relative humidity, with most conidia being produced at a relative humidity of 91.2 per cent.

There was a difference in the range for the production of cleistothecia in the two species: the range in *M. nitida* was from 100 through 29.5 per cent, and that in *M. brachytricha* was from 100 through 49.0 per cent, with an optimum production at 91.2 per cent, which is the optimum for growth and for the formation of conidia.

Sector mutants occurred in several of the plates at 70.4 per cent relative humidity and above, the most noteworthy of which occurred in cultures of *M. brachytricha* at 80.6 per cent relative humidity—a black mycelium which has continued true to type for over three years. Humidity, however, is not thought to be the deciding factor in the production of sector mutants, excepting that a high humidity favors rapid vegetative growth.

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THE OCCURRENCE AND TYPES OF VESSELS IN THE VARIOUS ORGANS OF THE PLANT IN THE MONOCOTYLEDONEAE¹

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APPARENTLY NO broad survey has been made to ascertain either the occurrence or the types of vessels present in the Monocotyledoneae. Indeed, the only paper of any real importance in this respect is one by Caspary published in 1862. The publications of Schulze (1893), Bädcker (1903), Bernatsky (1904, 1906), and Fuchsig (1911) also seem to report vessels authoritatively. Numerous publications have appeared in which the general topography of the vascular tissues in many groups of plants has been discussed, but in which only incidental observations concerning the true nature of the tracheary elements have been recorded. Papers of this nature, to mention only a few, are those by Scharf (1892), Colozza (1898, 1901), Plowman (1906), Chrysler (1906, 1907), Zweigelt (1913), and Bouvier (1915). Furthermore, the loose use of the words *Gefasse*, *vaisseau* and *vessels* by many writers has led to great confusion regarding the meaning of the term "vessel." These words have often been employed indiscriminately in describing tracheary elements, without any apparent attempt to determine the actual structure of such elements. Consequently, the observations recorded in the literature are often untrustworthy. In their review of the comparative anatomy of the Monocotyledoneae, however, Solender and Meyer (1928, 1929, 1930, 1933) draw a clear distinction between tracheids and vessels, but the information in this important series of publications is fragmentary. Generalizations concerning the occurrence and types of vessels in the Monocots, such as those made by Jeffrey (1917), are also apparently based on an unrepresentative section of the plants in this group of the angiosperms.

It is obvious from the foregoing that the present paper is a report on the occurrence and types of true vessels in the various organs from a wide representation of the Monocots. Other papers to follow will discuss a number of features of vessels, and will attempt to point out the lines of specialization these elements exhibit in this group of plants. The impressive evolutionary document developed from a consideration of the variations in tracheids and vessels in other groups of plants has assumed an increasingly important part in phylogenetic studies (see papers by Heimsch and Wetmore, 1939; Tippe,

1938; and Vestal, 1937). A study of these elements from the same viewpoint in the Monocots may very well produce results of importance along similar lines. Furthermore, an analysis of the data accumulated from all parts of the plant in genera and species from extremely diverse habitats has great possibilities for an *expansion* of, and a *check* on, the evidence concerning (1) the phylogenetic importance and (2) the physiological significance of the structural differences in tracheary elements.

A preliminary report (Cheadle, 1939) on this subject has been presented, at which time about one-third of the species now available were considered.

MATERIALS AND METHODS.—About 325 species of 220 genera in 41 families as defined by Hutchinson (1934) were investigated in the present study.² The names of the families are listed throughout the paper, and representative genera and sometimes species are noted under these families. Wherever possible, all parts of the plant were examined by the utilization of both sectioned and macerated tissues. Practically all tissues were macerated, but many of them have not yet been sectioned. The sections were made from materials embedded either in paraffin or in celloidin, following in general the techniques suggested respectively by Zirkle (1930) and Wetmore (1932). Materials of the Palmae and plants of similar construction generally need prolonged treatment with hydrofluoric acid and very gradual infiltration with celloidin. The method of preparing tissues for separation of the cells was based upon a variation of the Jeffrey method (Chamberlain, 1932). This variation includes the use of a constant temperature (55-60°C.) oven and a method for preventing the corks from blowing out of the bottles in which the material is placed during the maceration procedure. The clamps illustrated by Wetmore (1932) were used for this purpose. Experience alone dictates not only the percentage of chromic and nitric acids to be used with different materials but also the length

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of time necessary to keep the materials in the oven. A fair average for the materials treated would be the use of equal portions of 5 per cent nitric and 5 per cent chromic acids for a period of about twelve hours. Separation of the tracheary cells in a majority of tissues in the Monocots is difficult for two reasons. First, only a small number of vascular elements can be obtained from large pieces of material. Secondly, the walls of the elements are often too thin to withstand shaking about in a test tube, and the vascular elements are often surrounded by fibers. Therefore, the tissues must merely be softened in the maceration fluid and then later, after thorough washing, they must be teased out carefully on a slide. These separated cells, still on the slide, were stained in safranin, dehydrated, and finally mounted in diaphane.

Some organs were also injected with diluted black India ink to check observations made on macerated and sectioned tissues. The organs to be injected

were washed thoroughly in running water and pumped free of air. The diluted ink was placed in a 50 cc. burette, at the bottom of which was attached a piece of rubber tubing of proper diameter. The tubing was shut off by a screw clamp while the material was inserted. This insertion was carried out under water to prevent the introduction of air bubbles. The screw clamp was then loosened and the ink allowed to flow into the material. When viewed against a white background, the ink, if vessels were present, could be seen streaming into the water from the vascular bundles. The material was taken out after a period of fifteen or twenty minutes and then macerated as pointed out in the preceding paragraph. The presence of carbon in a linear succession of tracheary elements was considered as proof that vessels are present, even if the ink could not be observed streaming from the vascular bundles during the ink injection process. These injections were

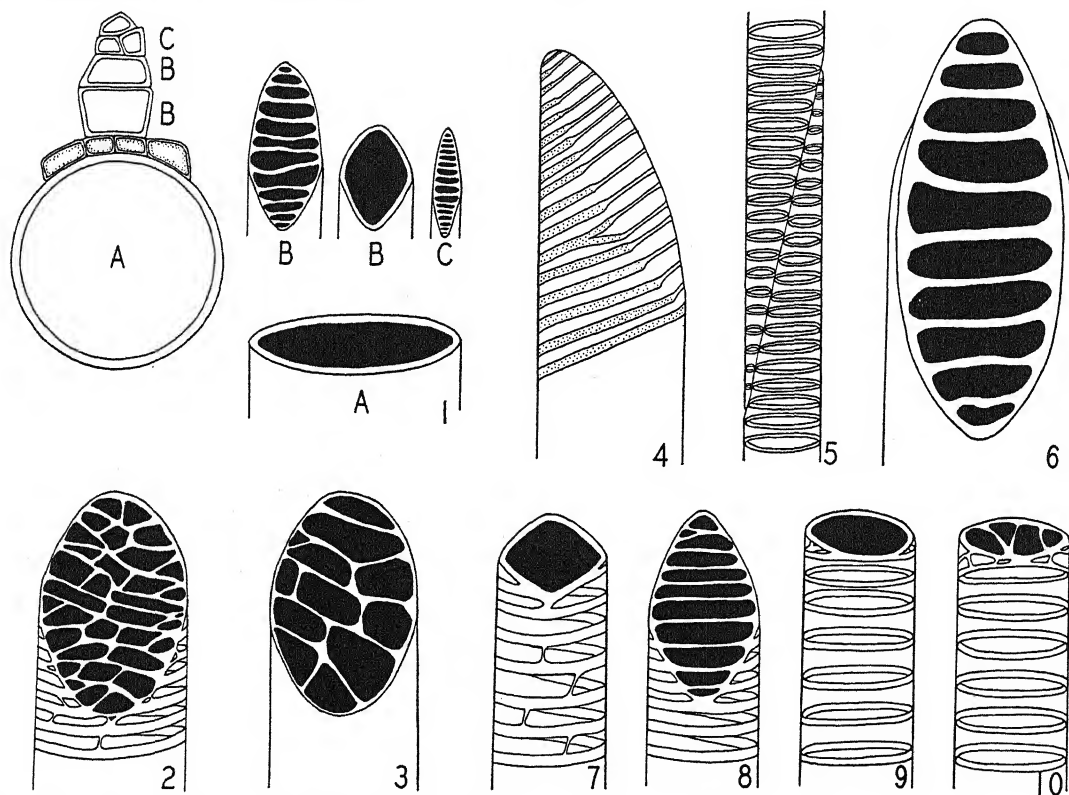


Fig. 1-10.—Fig. 1. *Butomus umbellatus* (Butomaceae), showing a pole of the xylem of root at left in cross section, and parts of vessels at right with perforation plates. The cross section of the large vessel was taken at the end of a vessel element, hence the apparent side wall is actually the perforation rim. The letters indicate the positions of the various vessels in the xylem pole. The last-matured metaxylem is at A, the earliest at C. $\times 500$.—Fig. 2. *Hymenocallis caribaea* (Amaryllidaceae), showing reticulate perforation plate of a vessel taken from center of the root. $\times 400$.—Fig. 3. *Cooperia pedunculata* (Amaryllidaceae), reticulate perforation plate of a vessel from center of root, a type rather common in the Amaryllidaceae. $\times 400$.—Fig. 4. *Dimerocostus uniflorus* (Zingiberaceae), portion of a tracheid from the stem, with an end wall which simulates a perforation plate. Tracheids in *Strelitzia* (Strelitziaceae) are similar. $\times 240$.—Fig. 5. *Carex intumescens* (Cyperaceae), parts of two annular tracheids where their ends overlap, taken from the stem. $\times 425$.—Fig. 6. *Phoenix dactylifera* (Palmae), a scalariform perforation plate taken from the stem. $\times 140$.—Fig. 7, 8, 9, 10, all from the stem of *Rhoeo discolor* (Commelinaceae). Fig. 7 is a spiral vessel with porous perforation plate, 8 is a spiral vessel with scalariform plate, 9 is an annular vessel with porous perforation plate, and 10 is an annular vessel with a reticulate perforation plate. All $\times 290$.

always repeated at least twice, and often more than three or four times in questionable cases.

Except for part of figure 1, the drawings were made freehand from macerated tissues.

OBSERVATIONS AND DISCUSSION.—Before proceeding to a discussion of the data, it should be emphasized that the final data used for this paper were often based on material from several plants or from several similar organs of the same plant. For example, several roots (adventitious) from a single grass plant, or several roots obtained from different specimens, were observed in ascertaining the true picture of the tracheary elements in a single species. Furthermore, in the stems of palms (and plants of similar structure), materials were examined from both the central and the peripheral regions at several levels, including areas near the ground level and near the crown of leaves. Various parts of the rachis in large palm leaves, as well as various parts of spreading inflorescence systems, received comparable attention. Similarly, whenever roots of small and large diameter were present in the same plant both were treated. In other words, an attempt was made wherever possible to obtain a representative sampling of the organs, whatever their size might be. This type of sampling, although it involves a large amount of routine work, has led to the conclusion that the variations that occur between different areas of any particular organ of a species, such as the stem of a palm, are so small as to be entirely negligible in a survey of this particular nature.

The discussion of the data has been divided so that it includes (1) families whose members have no vessels and have little or no thickening of the xylem elements, (2) families whose members have vessels only in the roots, (3) families which have both (a) members with vessels only in the roots and (b) members with vessels in the roots and also in some other organs of the plant, and (4) families with vessels throughout the plant. The investigation is primarily concerned with metaxylem elements, but wherever the protoxylem is not specifically mentioned, no protoxylem vessels were definitely identified. It is more than probable that, where both metaxylem and protoxylem are clearly present and vessels do not occur in the metaxylem, they are also absent from the protoxylem. This comment above is a preliminary statement on a definite problem which will be considered in a later paper. Environmental influences will also be considered in subsequent publications, although they will be more or less casually treated in a few of the families included in this paper.

The figures in the tables presented in this paper are based upon species rather than genera, because there is no evidence available at this time to show that all the species of a genus are anatomically similar with regard to the types of vessels present. As a matter of fact, in those genera in which several species have been examined, the species more often than not were found to vary among themselves. Within certain single genera in the grasses and sedges there is as great a variety of types of vessels as can be dis-

covered throughout the entire family. In only three cases were more than five species in any genus considered and the greatest number for any genus was eight.

The vessel will be discussed generally only in terms of the type of perforation plate which occurs on the vessel members. The term perforation plate and its variations used here (usually scalariform and simple or porous [Fig. 1, B and C]) are used in the sense in which they were defined by the Committee on Nomenclature of the International Association of Wood Anatomists (1933). The terms protoxylem and metaxylem are used as they are defined by Eames and MacDaniels (1925). The meaning of the terms early-formed (or matured) and later-formed metaxylem is indicated in figure 1 (and its legend).

Families whose members have no vessels and have little or no thickening in the xylem elements.—Hydrocharitaceae: *Elodea canadensis* Michx., *Vallesneria spiralis* L.; Lemnaceae: *Lemna perpusilla* Torr., *Spirodela polyrhiza* (L.) Scheid.; Najadaceae: *Najas flexilis* (Willd.) Rostk. & Schmidt., *N. flexilis* var. *robusta* Morong., *N. gracillima* (A. Br.) Magnus; Ruppiaceae: *Ruppia maritima* L.; Zannichelliaceae: *Zannichellia palustris* L.; Zosteraceae: *Zostera marina* L. All these families are in general composed of aquatics. In *Elodea* of the Hydrocharitaceae, annular to spiral tracheids occur in the nodes of the stems. In *Spirodela* of the Lemnaceae, annular tracheids are present in the roots and leaves. No vascular elements with even annular thickenings were found anywhere in the plant in the remainder of the species examined in this study, although Chrysler (1906) reported that "xylem" occurs in the nodes of the creeping stem of *Zostera* and that annular to spiral tracheids occur in the nodes of *Ruppia*, *Zannichellia*, and in *Najas flexilis*. The differences in observations may be due either to difference in age of the materials or to variability in the specimens themselves. At any rate, there appears to be no question concerning the absence of vessels.

Families with vessels in the roots only.—Table 1 includes a representation of the material discussed in this section.

Agavaceae: nine genera, twenty-four species, including *Agave*, *Cordyline*, *Dracaena*, *Nolina*, *Yucca*, etc. In all but eight species, only vessels with simple (porous) perforation plates were observed. In four species, a few scalariform plates were found in the early-formed metaxylem, while in one the scalariform and simple plates were more or less evenly divided. In three species, only scalariform plates are present. The dominant type of perforation is obviously the simple form. Vessels with scalariform plates were found in the leaves of *Cordyline* and *Dracaena*. Ink injections confirmed observations made of macerated and sectioned materials. Similar treatment of the stems in those genera indicated complete absence of vessels; the tracheids have scalariformly pitted end walls.

Alismataceae: *Sagittaria*, with four species and one forma. Simple perforations are the rule, with a

TABLE 1. Families having vessels in roots only. The variations in types of perforation plates and the number of species in which each type occurs.

Family	Total no. spp.	Perforation plates				
		Porous only	Porous and scanty sca- lariform	Porous and scalariform	Scalariform and scanty porous	Scalariform only
Agavaceae	24	17	4	1	0	2
Alismataceae	4	1	2	1	0	0
Alstroemeriaceae	1	1	0	0	0	0
Amaryllidaceae	20	1	1	0	0	18
Araceae	8	0	0	0	0	8
Butomaceae	2	0	1	1	0	0
Cannaceae	1	0	1	0	0	0
Hypoxidaceae	1	0	0	0	0	1
Juncaginaceae	1	0	0	0	0	1
Pontederiaceae	3	0	0	0	0	3
Potamogetonaceae	5	0	0	0	0	5
Scheuchzeriaceae	1	0	0	0	0	1
Strelitziaceae	3	0	0	0	0	3
Trilliaceae	3	0	0	0	0	3

few scalariform plates in the early-matured metaxylem. The stems of *Sagittaria* have tracheids with scalariformly pitted end walls, and the membranes remain in the pits.

Alstroemeriaceae: *Alstroemeria aurantiaca* D. Don. Only simple plates occur in the roots. The metaxylem of the stem has tracheids with scalariformly pitted, long oblique end walls.

Amaryllidaceae: fifteen genera, twenty species, including *Amaryllis*, *Clivia*, *Cooperia*, *Crinum*, *Narcissus*, etc. Scalariform plates with variations to reticulate (fig. 2 and 3) were found in fourteen genera and eighteen species. There are a few scalariform plates in the early-matured metaxylem in *Allium*, but the remainder of the vessels have simple plates. There is no question about the absence of vessels in the remainder of the plant, and the tracheids seldom have heavily thickened walls. These tracheids are usually reticulately thickened, or have thickenings so laid down as to leave conspicuous scalariform pits.

Araceae: seven genera, eight species, including *Acorus Calamus* L. and *Arisaema triphyllum* (L.) Schott. All these species have scalariform perforation plates. The stems of several (including *Acorus Calamus* L.) were injected with India ink to confirm the absence of vessels. The tracheids are often of large diameter and great length, and generally have scalariformly pitted side and end walls. The corm of *Arisaema* has numerous oval-pitted tracheids.

Butomaceae: *Butomus* and *Hydrocleis* with two species. In *Butomus* there are a few scalariform plates in the small (fig. 1, B and C) early-formed metaxylem, while in the remainder (fig. 1, A and B) the vessels have simple plates. In *Hydrocleis*, both scalariform and simple plates are found in the later-formed metaxylem, but scalariform alone occur in the early metaxylem. The tracheids in the remainder

of the plant are often similar to the vessel members, but membranes are present in the scalariform pits of the end walls.

Cannaceae: *Canna indica* L. The vessels have both porous and scalariform plates, the former occurring almost entirely in the early-formed metaxylem. The pitting in the tracheids of the rhizome and aerial stem is similar to that of the vessels in the roots, and the end walls of successive tracheids are arranged so as to resemble perforation plates. Although it is possible that some perforations actually may be present in these end walls, none was discovered.

Hypoxidaceae: *Hypoxis hirsuta* (L.) Coville. The roots have scalariform plates in the vessels. The length of some of these plates is not more than twice the diameter of the vessels. Scalariformly pitted tracheids are found in the corm and inflorescence axis. There are clearly no vessels in the shoot system in the material observed.

Juncaginaceae: *Triglochin maritima* L. This plant, collected along the coastal flats, has very long scalariform plates in the vessels of the roots. No vessels occur elsewhere, although scalariformly pitted tracheids (irregular in shape in the rhizome) are present throughout the plant.

Pontederiaceae: *Pontederia* and *Eichornia* with three species. Only scalariform plates occur in the roots. The stems of *Eichornia crassipes* Solms have elements whose end walls are very similar to scalariform perforation plates.

Potamogetonaceae: *Potamogeton* with five species. Clear perforation plates of rather short length were seen at least in the larger vessels of the roots. Vascular elements in the shoot system are in general merely protoxylic in nature and are clearly not vessels. Those metaxylic elements which occur are scalariformly pitted and are without doubt tracheids.

Scheuchzeriaceae: *Scheuchzeria palustris* L. Long scalariform plates (the pit membranes may, how-

ever, remain undissolved in some cases) occur at least in the last-matured metaxylem of the roots, and very long tracheids appear in the remainder of the plant. The latter elements are not strongly thickened and the end walls sometimes have the appearance of perforation plates.

Strelitziaceae: *Heliconia*, *Ravanelia*, *Strelitzia*, with three species. Only scalariform plates occur in the roots. Large protoxylem elements generally occur in the shoot, and these annular to spiral elements have long end walls. That is, these cells gradually taper down to very small diameters. In the inflorescence axis of *Strelitzia*, the spiral elements are often characterized by shorter, but obliquely placed, end walls whose bars of thickening are somewhat smaller than those of the remainder of the element (fig. 4). Ink injections, however, indicated that vessels are absent. A few metaxylem tracheids, smaller in diameter, are also present in the shoot system, although the leaves generally lack them.

Trilliaceae: *Trillium* and *Medeola*, with three species. The scalariform plates in the roots find no counterparts in the remainder of the plant. Metaxylem elements are generally scalariformly pitted and have no end walls suggestive of perforation plates.

Families which have both (a) members with vessels only in the roots and (b) members with vessels in roots and also in some other organs of the plant.—Table 2 gives a representation of the material discussed in this section. Since the tabular presentation of the data from all parts of the plant in this particular category of families is somewhat difficult, only the roots, stems, and leaves are included. If the inflorescence axis is the only real aerial stem structure, as is true in *Musa*, it is included as a stem. Where vessels occur in other organs in the plant, they are mentioned in the treatment of the separate families.

Bromeliaceae: six genera, eight species, including *Billbergia*, *Dyckia*, *Tillandsia*, etc. Only scalariform plates occur in the roots of the four species studied. The material available from the shoot system was not complete enough to make an adequate comparison between the xylem of the roots and the shoots. Furthermore, the elements in most of the plants examined are small in diameter and, therefore, difficult to work with. In only one of the stems of the six species observed are vessels clearly absent. In the remaining five, vessels are questionably present. At least the end walls of the elements are significantly different from the side walls in pitting, and probably some pit membranes are lost to form perforation plates of the scalariform type. Of the leaves examined in four species, three clearly have no vessels, while in one scalariform plates without doubt occur. This family seems, therefore, to have either very primitive perforation plates in both the stems and roots, or merely in the roots.

Iridaceae: *Gladiolus*, *Iris* and *Sisyrinchium*, with five species. In the roots of all these species the prevailing type of perforation plate is the simple form. In all of the roots, however, a few scalariform plates

occur in the early-matured metaxylem. No vessels occur in the shoot system of *Iris* and *Gladiolus*, and the tracheids are reticulately or scalariformly thickened throughout. In *Sisyrinchium angustifolium* Mill., however, scalariform plates are found throughout the shoot system. These occur in the metaxylem, and are usually limited to the larger elements. There may be, of course, other plants with vessels throughout in this family.

Liliaceae: thirty-one genera, forty-three species, including *Aloe*, *Asparagus*, *Clintonia*, *Colchicum*, *Convallaria*, *Lilium*, *Polygonatum*, etc. There is considerable variation in this family, both with regard to the presence or absence of vessels and to the type of perforation plates in those vessels which are present. The roots of this family generally have scalariform plates in the vessels, but *Aloe*, *Anthericum*, *Asparagus*, *Chlorophytum*, *Kniphofia*, *Liriope*, *Ornithogalum*, *Paradisea*, and *Smilacina* generally have porous plates, with a few scalariform plates in the early-matured metaxylem in some cases. The remainder of the total number noted in table 2 under the column of both porous and scalariform have only porous plates in the metaxylem which is the very last to mature. Only in *Chlorophytum elatum* R. Br. do vessels occur throughout the plant, with scalariform plates in the stems and leaves. *Asphodelus* also has vessels in the stems, but no leaves were available for study. In *Asparagus*, scalariform plates are present in the metaxylem of the aerial stems, but vessels are absent in the rhizomes and in the modified stems which serve as leaves. Vessels with scalariform plates occur in the aerial stems of *Polygonatum commutatum* (R. & S.) Dietr., but are absent elsewhere in the shoot system. Ink injections confirm this statement. Ink injections were also made of the stems and rhizomes of *Clintonia*, *Convallaria*, *Lilium*, *Smilacina*, and *Uvularia* to confirm the absence of vessels in these organs. The tracheids in the plants just mentioned are long, but have end walls which to a certain extent simulate perforation plates. However, membranes are apparently present in the pits of the scalariformly thickened end walls in these tracheids. At best, the material investigated indicates that vessels seldom occur in stems of these genera. In the great bulk of the Liliaceae there are no vessels in the shoot system, although there are vessels of some type in all of the roots.

Marantaceae: *Calathea*, *Maranta*, *Thalia*, with four species. Aside from *Maranta leuconeura* Morr., no complete plants were available. Scalariform plates were found in the roots and rhizomes of this species but not elsewhere in the plant. No vessels occur in the stems of *Calathea*. In *Thalia*, spiral and scalariform elements of the stems and leaves have long obliquely placed end walls whose bars of thickening are narrower than those of the side walls. Some of these, at least, are probably vessels, although not enough material was available to confirm this conclusion by the use of ink injections.

Musaceae: *Musa paradisiaca* L. and *M. paradisiaca* var. *sapientum* Kuntze. Vessels in the roots

TABLE 2. Families with both (1) members with vessels in roots only and (2) members with vessels also in some other organ of plant. Indicating presence or absence of vessels, the type of perforation plates, and number of species in which each occurs.

Family	Total spp. ^a	Perforation plates in roots			Occurrence of vessels		
		Porous only	Scalariform and porous	Scalariform only	In stems ^b	In stem and ^b leaf of same species	In roots only
Bromeliaceae	8	0	0	4	5? in 6	1 in 4	1 in 2 ^d
Iridaceae	5	0	5	0	1 in 5	1 in 5	4 in 5
Liliaceae	43	5	14	23	7 in 41	1 in 41	35 in 41
Marantaceae	4	0	0	1	1 in 4	0 in 3	0 in 1
Musaceae	2	0	1	0	1 in 1	0 in 1	0 in 1
Orchidaceae	20	0	1	3	5 in 16	1 in 9	3 in 4
Ruscaceae	2	0	0	1	1 in 2	0 in 1	1 in 1
Sparganiaceae	5	0	0	3	3? in 3	3 in 3	0 in 3
Zingiberaceae	14	0	0	4	7? in 10	4 in 8	3 in 3

^a Since not all organs of the plant in all species were available, the total species merely indicates the total in which at least one organ was available.

^b Only scalariform plates occur in these organs.

^c The question mark indicates that vessels are questionably present.

^d The totals in this column are usually low because the roots, stems, and leaves of any species must be available in order to construct the column.

have simple plates in later-matured metaxylem and scalariform plates generally in the early-matured metaxylem. Scalariform plates seem to occur in the inflorescence axes but apparently nowhere else in the shoot system. The larger elements in the xylem are the vessels and these have scalariformly pitted side walls which are weakly thickened. Only spiral and annular tracheids occur in the leaves studied.

Orchidaceae: fifteen genera, twenty species, including *Calopogon*, *Cattleya*, *Cypripedium*, *Dendrobium*, *Vanilla*, etc. Only scalariform plates are found in the roots of three of the four species studied, and a few simple plates occur with the scalariform ones in the remaining species. This family apparently has considerable variation with respect to the presence or absence of vessels in the shoot system. The larger elements of the metaxylem of the stems in five spe-

cies, especially in *Vanilla*, are undoubtedly vessels with scalariform plates. Vessels questionably occur also in two other species. In *Calopogon*, *Cypripedium*, *Dendrobium*, *Habenaria*, and *Renanthera* vessels are definitely absent in the stems (and inflorescence axes). Vessels with scalariform plates occur in the leaves of *Epidendrum* and *Scaphyglottis*, but could not be identified with any degree of certainty in the leaves of other species. The tracheids in this family are numerous in each bundle and generally have considerable wall thickening, although spiral and annular forms also occur in all cases. Further material of this large family is needed to ascertain the degree of variation in the occurrence and specialization of vessels.

Ruscaceae: *Ruscus aculeatus* L. and *Danae racemosa* Moench. *Ruscus* has scalariform plates in the

TABLE 3. Families with vessels throughout the plant in all members. Indicating the

Family	Total number species studied	Roots			Underground stems		
		Perforation plates			Perforation plates		
		Porous only	Porous and scalariform	Scalariform only	Porous only	Porous and scalariform	Scalariform only
Commelinaceae	5	0	4	0	—	—	—
Cyperaceae	31	0	27	0	1	21	1
Dioscoriaceae	3	—	—	—	—	—	—
Eriocaulaceae	1	0	0	1	0	0	1
Gramineae	45	20	19	0	8	7	0
Haemodoraceae	2	0	2	0	0	0	1
Juncaceae	10	1	8	0	1	7	0
Palmae	26	0	21	0	—	—	—
Pandanaceae	3	0	0	2	—	—	—
Smilacaceae	7	0	0	3	0	0	1? ^a
Typhaceae	2	0	0	2	0	0	2
Xyridaceae	2	2	0	0	1	0	0

^a The question mark indicates that vessels are questionably present.

roots and there are no vessels elsewhere in the plant. *Danae* has scalariform plates in the stems, but no further organs of this plant were available for study. The large vessels of *Danae* are quite in contrast to the very small and numerous tracheids of *Ruscus*.

Sparganiaceae: *Sparganium*, with five species. Well-defined scalariform plates occur in the vessels of the roots. In forms like *S. lucidulum* Fernald & Eames, vessels with long scalariform plates are clearly present throughout the plant. In *S. fluctuans* (Morong.) Robinson, vessels are absent in the rhizome. Scalariform plates are probably present in all other species and organs, although they are not so clearly defined as in *S. lucidulum*.

Zingiberaceae: nine genera, fourteen species, including *Alpinia*, *Amomum*, *Costus*, *Hedychium*, *Languas*, etc. The roots of the four species available all have scalariform plates. In seven of the ten species in which aerial stems (or inflorescence axes) were available, vessels with long scalariform plates are probably present. These elements do not have such sharply outlined plates as occur in the roots, but they are significantly different from the side walls. In three species, vessels are definitely absent in the stems. The leaves in five of the nine species have no vessels, while the remaining four have long scalariform plates similar to those in the stems. The tracheids and vessels in this family are generally thin-walled and often have spiral thickening (fig. 4) with an occasional connecting bar across the spiral. The conducting elements are, therefore, difficult to macerate successfully. There seems to be little doubt that this family has (1) species with vessel-less shoot systems, as well as (2) species with vessels throughout the plant, and (3) species with vessels in the root and stem only.

Families with vessels throughout the plant.—Table 3 does not indicate the relative number of porous and scalariform plates under the column "porous and scalariform". The data were originally charted to indicate "porous and scanty scalariform" and "scalariform and scanty porous", in addition to

the columns indicated in the table. This analysis of the data will be given for those families in which such a treatment seems pertinent.

Commelinaceae: *Commelina*, *Rhoeo*, *Tradescantia*, *Zebrina*, with five species. The roots of these species all have porous plates in addition to a few scalariform plates in the early-matured metaxylem. In the aerial stems, the porous and scalariform plates occur in about equal numbers in three species while in the remaining species mostly porous plates occur. The inflorescence axes and leaves generally have equal mixtures of the two forms of plates, the only exception being that the leaves in one of the four species have more scalariform than porous plates. The vessels in the roots have scalariform to reticulate thickenings on the walls, while the thickenings on these elements of the shoot system are often spiral only, and may even be annular. The clearest examples of loosely spiral to annular vessel elements were seen in this family, especially in the stems of *Tradescantia* and *Rhoeo* (fig. 7, 8, 9, and 10).

Cyperaceae: eleven genera, thirty-one species, including *Carex*, *Cyperus*, *Dulichium*, *Eleocharis*, *Eriophorum*, *Scirpus*, *Scleria*, *Stenophyllus*, etc. (representatives of all tribes in Gray's Manual, 1908). The roots in twenty-one species of this family have porous plates with rare scalariform plates in the early-matured metaxylem. In six species there is a more common occurrence of scalariform plates. In the rhizomes, these numbers are respectively 7 and 11, with one species having porous plates only and one having scalariform plates only. In the aerial stems, the numbers are respectively 5 and 23, with one species having scalariform plates only. The numbers in the inflorescence axes are 1 and 9, with one species having porous plates only. Similarly, the numbers in the leaves are 1 and 21, with one species having scalariform plates only. These numbers indicate that the porous plate is most common in the roots and becomes less common as the remainder of the plant is considered in succes-

number of species and the organs in which the various types of perforation plates occur.

Aerial stems			Inflorescence axes			Leaves		
Perforation plates			Perforation plates			Perforation plates		
Porous only	Porous and scalariform	Scalariform only	Porous only	Porous and scalariform	Scalariform only	Porous only	Porous and scalariform	Scalariform only
0	5	0	0	2	0	0	4	0
0	28	1	1	10	0	0	22	1
0	0	3	—	—	—	—	—	—
0	0	1	0	0	1	0	0	1
15	30	0	9	18	0	11	32	0
0	0	2?	0	0	1	0	0	1?
0	10	0	0	8	0	0	6	1
0	8	15	0	0	6	0	0	22
0	0	2	—	—	—	0	0	1
0	0	7	—	—	—	0	0	4
0	0	2	0	0	2	0	0	2
2	0	0	—	—	—	2	0	0

sion up to the leaves. The contrary is true of scalariform plates. This general conclusion is true even when the more aquatic types are considered alone, just as it is when the more terrestrial members of this family are treated alone. In other words, the variation that may occur in the same organ in any representative group of species obtains, whether the group is generally more aquatic or generally more terrestrial. No annular or spiral vessels were observed in this family, although further work will be necessary to determine whether any of the elements of this type are actually vessels. Undoubtedly many of them are tracheids, for elements with long tapering ends (fig. 5) and all membranes intact were often successfully separated from the remainder of the xylem.

Dioscoreaceae: *Dioscorea*, with three species. It is perhaps unjustifiable to place this family among those which have vessels throughout the plant, for only aerial stems were available for study. These have very broad vessels with long scalariform plates which often exhibit branching of the bars in the plate.

Eriocaulaceae: *Eriocaulon articulatum* (Huds.) Morong. Vessels with scalariform plates occur throughout the plant in this species, the plates being shorter and less oblique in the roots and rhizomes than they are elsewhere. Furthermore, only the larger elements of the metaxylem in the stems and leaves are clearly vessels. The remainder are tracheids.

Gramineae: thirty-three genera, forty-five species, including the common cereals, members of all tribes as defined by Hitchcock (1935) for grasses in the United States, and a few sub-tropical species. As in the Cyperaceae, the data in table 3 do not adequately demonstrate the true distribution of the perforation plates in the organs of this family. The roots in twenty species have only porous plates. Porous plates with rare scalariform plates in the early-matured metaxylem occur in seventeen species, while in only three species are scalariform plates in any sizeable number present together with the more numerous porous forms. In the rhizomes these numbers are respectively 8, 6 and 1, while in the aerial stems the numbers are 15, 27 and 3. Similarly, it should be stated that in the inflorescence axes, nine species have porous plates only, fourteen have porous plates with rare scalariform plates, and four have a considerable number of scalariform plates together with the porous type. In the leaf, the numbers are respectively 11, 22 and 10. It will be noted that the roots lead in the total number of porous plates and that the leaves are last in this respect. The situation is similar to that in the Cyperaceae, the only difference being that porous plates appear in greater numbers in the Gramineae. The same general distribution seems to occur in all parts of the family, whether the species are from aquatic, mesophytic, or xerophytic habitats. The ratio of porous plates to scalariform plates is the same in corn as it is in wild rice and the same in various bamboos as it is in *Bou-*

teloua gracilis (H. B. K.) Lag. In *Zea*, *Triticum*, and *Avena*, as in practically all other grasses studied, the elements directly above the protoxylem and between the two large pitted vessels of the metaxylem are vessels and not tracheids, as reported by Hayward (1938). This situation is true in both stems and leaves. Favorably macerated materials show that these elements often have perforation plates on oblique end walls where they are likely to be overlooked, even in thin sections. Spiral vessels, chiefly with porous plates, were identified in at least one organ of the shoot system in twenty-two species. They could not be identified with certainty in the roots. Such vessels may occur throughout the family but have been unidentifiable (1) because it is difficult to separate them out in macerations, and (2) because the materials were often not available in a stage of maturation in which these elements were not stretched completely out of their normal shape.

Haemodoraceae: *Lachnanthes tinctoria* (Walt.) Ell. and *Xiphidium coeruleum* Aubl. Porous plates occur in the later-matured metaxylem and scalariform plates in the early-matured metaxylem of the roots in these two species. Scalariform plates alone occur throughout the remainder of the plant, although the long end walls of the larger metaxylem elements in the stem of *Xiphidium* and in the leaf of *Lachnanthes* are not clearly defined perforation plates. No material of the leaf of *Xiphidium* was available.

Juncaceae: *Juncus* with eight species, and *Luzula* with two species. The data in this family, as in the Cyperaceae and Gramineae, may also deserve more detailed treatment than is afforded in table 3. Porous plates do not occur alone in the roots of these species. Porous plates with a few scalariform plates in the early-matured metaxylem occur in four species, and somewhat more equal numbers of porous and scalariform plates in three species. In one (*Juncus militaris* Bigel.), the scalariform type is more numerous than the porous and even occurs in the last-matured metaxylem. In rhizomes, these numbers are respectively one, five, one and one. Similarly, these numbers in the aerial stem are 0, 2, 4, and 4; in the inflorescence axes, 0, 1, 7, and 1; and in the leaves, 0, 1, 2, 3, with one species having scalariform plates alone. A study of these figures indicates that the porous perforation plate is most common in the roots and rhizomes, and least common in the leaves. The aerial stems and inflorescence axes are intermediate in this regard. The variation that occurs in the distribution of perforation plates obtains in general not only when the more aquatic members are considered as a group, but also when the more terrestrial forms are so treated, although there is a tendency for the porous form to occur in greater numbers in the more terrestrial species.

Palmae: twenty-two genera, twenty-six species, including *Adonidia*, *Archontophoenix*, *Caryota*, *Cocos*, *Elaeis*, *Kentia*, *Phoenix*, *Roystonea*, etc. Table 3 gives a clear enough picture of the variation among different organs, although investigations of

a more detailed nature (number of bars in the scalariform plates, for example) would reveal more differences. Twenty-one species in which the roots were examined have both scalariform and porous plates in these organs, although the scalariform plates almost invariably are restricted to the early-matured metaxylem. The stems in eight species have both porous and scalariform plates, while those in fifteen species have the scalariform type (fig. 6) alone. Scalariform plates alone occur in the inflorescence axes of the six species studied, as well as in the leaves of the twenty-two species examined. The perforation plates are also exclusively scalariform in the rachis of the leaf in the species studied. In the stems of a number of species, spiral elements with closely set thickenings were identified as vessels with scalariform plates. No such elements with porous plates were found, and no annular elements were identified as vessels.

Pandanaceae: *Freycinetia Banksii*, *Pandanus Veitchii* Dall., and *P. utilis* Bory. The parts available for study indicate that vessels with scalariform plates are found throughout the plant. Small, scalariformly pitted elements in the roots, stems, and leaves are tracheids.

Smilacaceae: *Rhipogonum*, *Smilax* (five species), *Heterosmilax*, eight species in all. Long scalariform plates occur in the metaxylem in all parts of the plant investigated, except in the rhizome of *Smilax herbacea* L., where only tracheids occur. The vessels tend to be quite broad (175 μ in *S. panamensis* Morong.), especially in the stems. The smaller elements of the metaxylem in this family are tracheids with scalariform pitting in the end walls.

Typhaceae: *Typha angustifolia* L. and *T. latifolia* L. Scalariform plates are found throughout the plant in these species. The plates are clearly defined in the roots, and less so in the shoot system where they are longer. Only the larger elements in the metaxylem of the latter are vessels, the remainder being tracheids. Ink injections confirm these statements.

Xyridaceae: *Xyris flexuosa* Muhl. and *X. Smalliana* Nash. Vessels with porous perforation plates occur in this family in all organs. The wall thickening varies from spiral to reticulate, even in the same vessel member, although the reticulate type is most commonly present. Vessels in the stems and rhizomes are likely to be more heavily thickened, often to the point where elongate pits are left on certain side walls at least.

CONCLUSIONS.—The conclusions in a paper of this nature may be expressed very briefly. Without doubt vessels are lacking in the shoot system of many species in the Monocotyledoneae. In some species vessels are absent throughout the plant. Where present, vessels may have either (1) scalariform (with variations to reticulate) perforation plates with varying numbers of cross bars or (2) simple plates. These observations may be summarized as follows:

1. Many aquatics are without vessels and are often devoid of normal xylem with its familiar tracheary elements.

2. Vessels occur in the roots of all species examined, with the exceptions noted above.

3. In many species, only the roots possess vessels.

4. A small number (twelve) of species have vessels in both the roots and the stems but not in the leaves.

5. The species mentioned in points three and four above may occur in families, as far as they have been investigated, in which other species have vessels throughout.

6. In the species of only two genera were vessels observed in the roots and leaves and not in the stems.

7. Many species have vessels throughout the plant, as in the Gramineae and Cyperaceae.

8. Vessels do not occur in the shoot system of typically bulbous or cormose plants, but are present in the roots.

9. A study of species (a) which have vessels in the root and stem and (b) which have vessels throughout the plant reveals, as earlier pointed out indirectly by the author (Cheadle, 1940), that simple perforation plates occur most commonly in the roots, least commonly in the leaves, and that they are intermediate in their occurrence in the aerial stems.

10. In those species whose leaves have vessels, the ratio of the scalariform plates to simple plates in the leaves is almost invariably greater than that in the stems and still greater than that in the roots.

11. The general distribution of the perforation plates described in the two preceding sentences occurs in species collected from practically all types of environment.

12. Both spiral and annular vessels (with either scalariform or simple plates) were identified in a number of species, especially in aerial stems, where vessels were likewise present in the metaxylem. In anticipation of papers to follow, it appears almost certain that, where both protoxylem and metaxylem are present and vessels are absent from the metaxylem, they are likewise absent from the protoxylem.

It may be stated again that the significance of (1) the presence or absence of vessels and of (2) the distribution of the various types of perforation plates will be discussed in subsequent work.

SUMMARY

A statement is made concerning the scope of the work of which this paper is a part.

The method of macerating tissues as modified for use in treating parts of the plant in the Monocots is described.

The number of genera and species examined, the presence or absence of vessels, and the types of perforation plates are indicated for all organs available in each of the forty-one families discussed.

Many aquatics are without vessels. Vessels occur in the roots of all species examined, with the exceptions noted above, and in many species vessels occur nowhere else in the plant. Typically bulbous and cormose plants have no vessels in the shoot system.

Vessels occur in the roots and stems in some species, but not in the leaves. The genera *Dracaena* and *Cordyline* have species in which vessels occur in the roots and leaves, but not in the stems. In some species vessels occur in all organs. Some families have members with vessels in the roots alone, other members with vessels in the root and stem, and still other members with vessels throughout the plant.

In species which have vessels in the root and stem or which have vessels in the root, stem, and leaf, simple perforation plates occur most commonly in the roots, least commonly in the leaves and are intermediate in their occurrence in the aerial stems. In those species whose leaves have vessels, the ratio of

the number of scalariform plates to the number of simple plates in the leaves is almost invariably greater than that in the stems and still greater than that in the roots. The general distribution of the perforation plates just described occurs in species collected from practically all types of environment.

Both spiral and annular vessels (with either scalariform or simple plates) were identified in a number of species, but only in those which also have vessels in the metaxylem.

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CYTOGENETICS OF A FACTOR FOR MULTIPLOID SPOROCTES IN BARLEY¹

Luther Smith

A CONSIDERABLE number of genetic factors have been found in various organisms which disturb the process of meiosis and thus contribute to the analysis of its mechanism and complexities. Descriptions of the irregularities wrought by a number of these factors and reviews of and citations to literature dealing with many others may be found in Darlington (1937), Smith (1936, 1939), and Clark (1940).

The present paper deals with the cytology and genetics of a recessive factor in barley (*Hordeum vulgare*, $n=7$) which affects meiosis in such a manner as to result in meiotic metaphases with 14, 21, 28, 56, 112, or various other numbers of pairs of chromosomes instead of the normal seven. This is apparently accomplished by the assembling of chromosomes from different cells rather than by the repeated division of chromosomes within individual cells.

MATERIALS AND METHODS.—Seed of the “multiploid sporocytes” (symbol *mu*) line was supplied in 1939 by G. A. Wiebe of the Bureau of Plant Industry from a selection out of C. I.² No. 3845, a six-rowed variety. (The mutant was formerly referred to as “contabescent anther.”)

Cytological observations were made on aceto-carmine smear preparations. Whole heads were fixed in Carnoy's fluid for a day or more; root tips for a week or longer. Some smears were made permanent by a tertiary butyl alcohol method described by Sears (1941). Most of the photomicrographs are from slides made permanent by this method.

CYTOLOGICAL OBSERVATIONS.—Typically at diakinesis the pollen mother cells (hereafter referred to as PMC's) have seven pairs of chromosomes as in normal barley though frequently the boundaries of individual cells are not clear. That is, the PMC's in an anther sac tend to form plasmodium-like masses of various sizes in which the chromosomes lie in groups (fig. 1). The numbers in the groups are not large and usually the seven bivalents belonging with a particular nucleolus can be identified.

At first meiotic metaphase the chromosomes form a plate in the PMC material. In some instances this material consists of only one PMC and in others consists of the aggregated cytoplasm of several microsporocytes. In some groups there are 7 pairs, in others 14, 21, 28, 35, 42, etc. As many as approxi-

mately 112 pairs have been counted in a single plate. Such a group is shown in figure 3 and may be compared with the normal as shown in figure 2. Larger groups have been observed, but the difficulty of counting such numbers is obvious. Some typical groups are shown in figures 4 and 5. It is apparent from figure 4 that the chromosomes may line up perpendicularly or horizontally in the tube of PMC material in the anther sac. It is also clear that more than one plate may form within a single mass of PMC material.

There is probably no particular limit to the number of pairs that might gather into a single group or plate. Chromosomes seem relatively free to move within the pollen mother cell material of an anther sac. Thus theoretically if all the PMC's formed into one mass all the bivalents within a locule could line up in a single metaphase plate. This is probably unlikely, but even if it occurred, such a mass would be broken up in making a smear preparation.

Quadrivalents are observed in some polyploid groups (fig. 6). These multivalents occur only in cells with 28 or more chromosomes. This indicates that some cells are sufficiently merged at the time of synapsis to permit pairing between chromosomes that would normally have been in separate cells. In figure 6 two open rings and one zigzag group of four are present. If a translocation were present, there should be about 17 quadrivalents, as there are approximately 238 chromosomes in this group (equivalent of 17 cells). Figure 3, which is from the same plant as figure 6, shows only bivalents; thus a reciprocal translocation is not present.

In addition to the mingling of chromosomes from different “cells” at an early stage, there is evidently a later migration of chromosomes in the formation of some of the polyploid metaphase plates. This is indicated by the concentration of chromosomes in parts of the PMC material, leaving the rest of it lacking in chromatin (fig. 4, 5), and by the greater aggregation of chromosomes at metaphase than at diakinesis. Still further evidence of this movement has been observed in the less complex groups of two cells incompletely fused. In some such instances the chromosomes from one cell have apparently crossed a connecting bridge and joined those in another (fig. 7). In other instances the chromosomes from the two cells have met at their mutual boundary. The connecting bridge between some cells appears so narrow that all the bivalents could not have traversed it simultaneously. The spindle of the merged plate may have a “pole” near the center of each of the two cells (fig. 7, lower right-hand corner), or it may be oriented at various angles to an imaginary line between the centers of the cells.

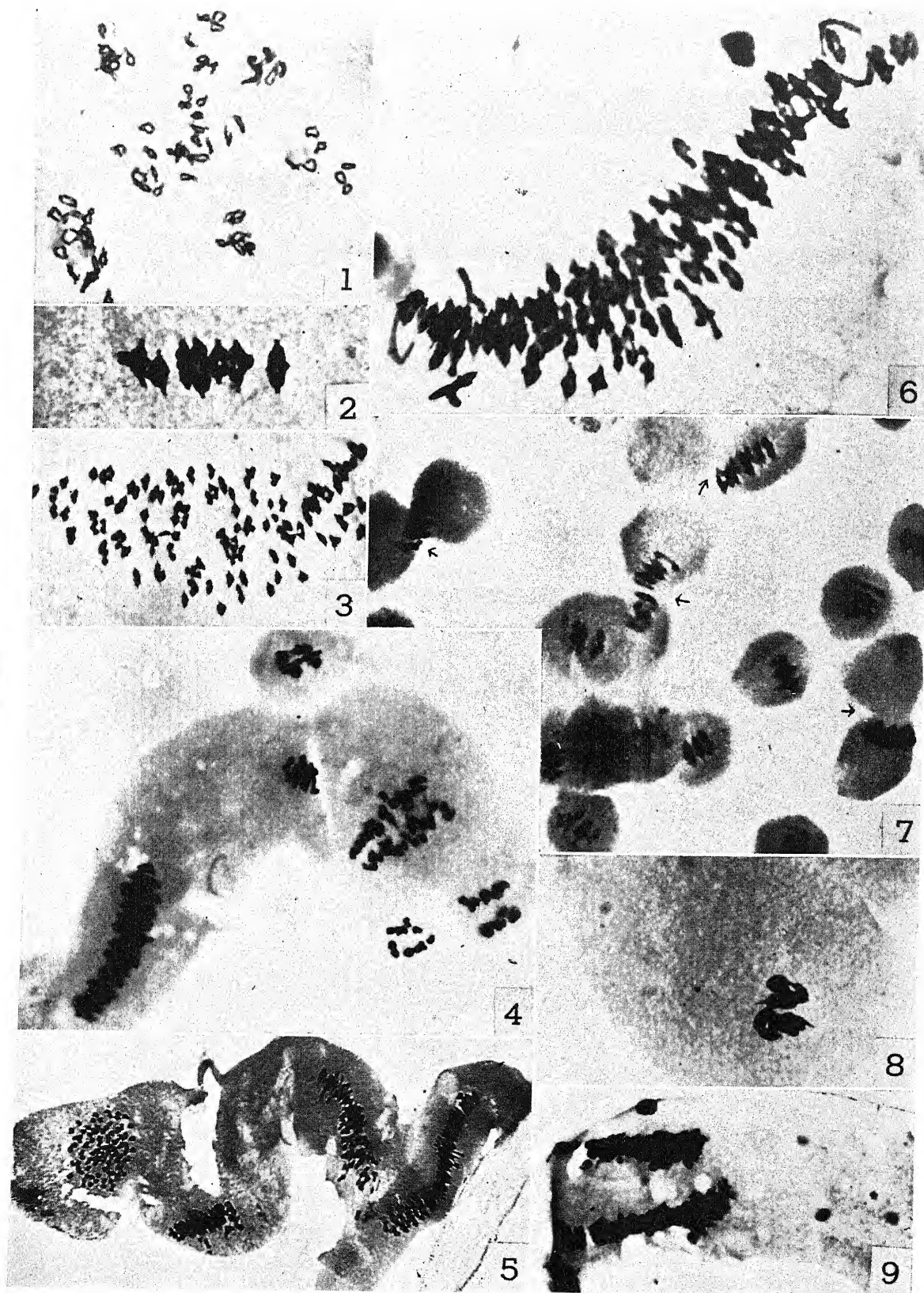
The movement of the chromosomes in forming the large groups is apparently through considerably greater distances for some of the bivalents than is

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² C. I. refers to accession number of the Division of Cereal Crops and Diseases.



ever accomplished in the formation of a plate in normal organisms.

One question which arose was whether the groups were always composed of seven or multiples of seven pairs, that is, whether the seven bivalents associated with a given nucleolus acted as a unit. Usually the larger groups are multiples of seven. Only a few have been observed which had other than seven or a multiple of seven pairs of chromosomes. One of these had 9, another 4 (fig. 8) and a third 16 or 17.

The foregoing is a description of the typical behavior, but there is some variability. Normal meiosis has been observed in anthers from the central florets of a spike, while large groups of chromosomes were found in anthers from lateral florets of the same spike. Even if the central florets exhibit the peculiar behavior, the groups of chromosomes tend to have 14 or 21 pairs rather than larger numbers. Meiosis in the central florets is less abnormal in plants grown in the greenhouse than in those grown in the field. Normal microsporogenesis has not been observed in the lateral florets. Thus the fusion (or incomplete separation) of PMC's varies from the occasional incomplete merging of two cells to the other extreme where there is only infrequently a cell which is not merged with several to many other cells.

First anaphase (and later stages) proceeds at about the same tempo in the groups with many chromosomes as in those with only 7 pairs (fig. 4). At late anaphase in the larger groups the chromosomes do not converge on a point. From the shape and size of the spindle in these groups it is doubtful if there is any point which functions as a "pole" in the usual cytological sense of the term. In this respect the spindle is similar to the divergent meiotic spindle in maize described by Clark (1940).

In many groups the spindle is obviously much shorter in proportion to its width than normal, i.e., the spindle is much wider, but little if any longer. It seems to extend only a short distance beyond the centromeres of the chromosomes regardless of how much PMC material may lie beyond or how many chromosomes are in the group. In anaphase the chromosomes move apart about the same distance as in normal plants, though the movement is frequently not limited by the bounds of the PMC material. Evidently the movement is stopped by some other mechanism, such as possibly the inability of a repelling force (electromagnetic, or protoplasmic streaming?) to push them further apart. The movement may be

limited by the termination of the spindle but, as pointed out above, in many instances there is no apparent obstruction to the further extension of the spindle.

At first telophase there is apparently a tendency for the chromosomes to form a spherical nucleus as in normal plants, but, if the number of chromosomes is large, an elongate nucleus is formed (fig. 9).

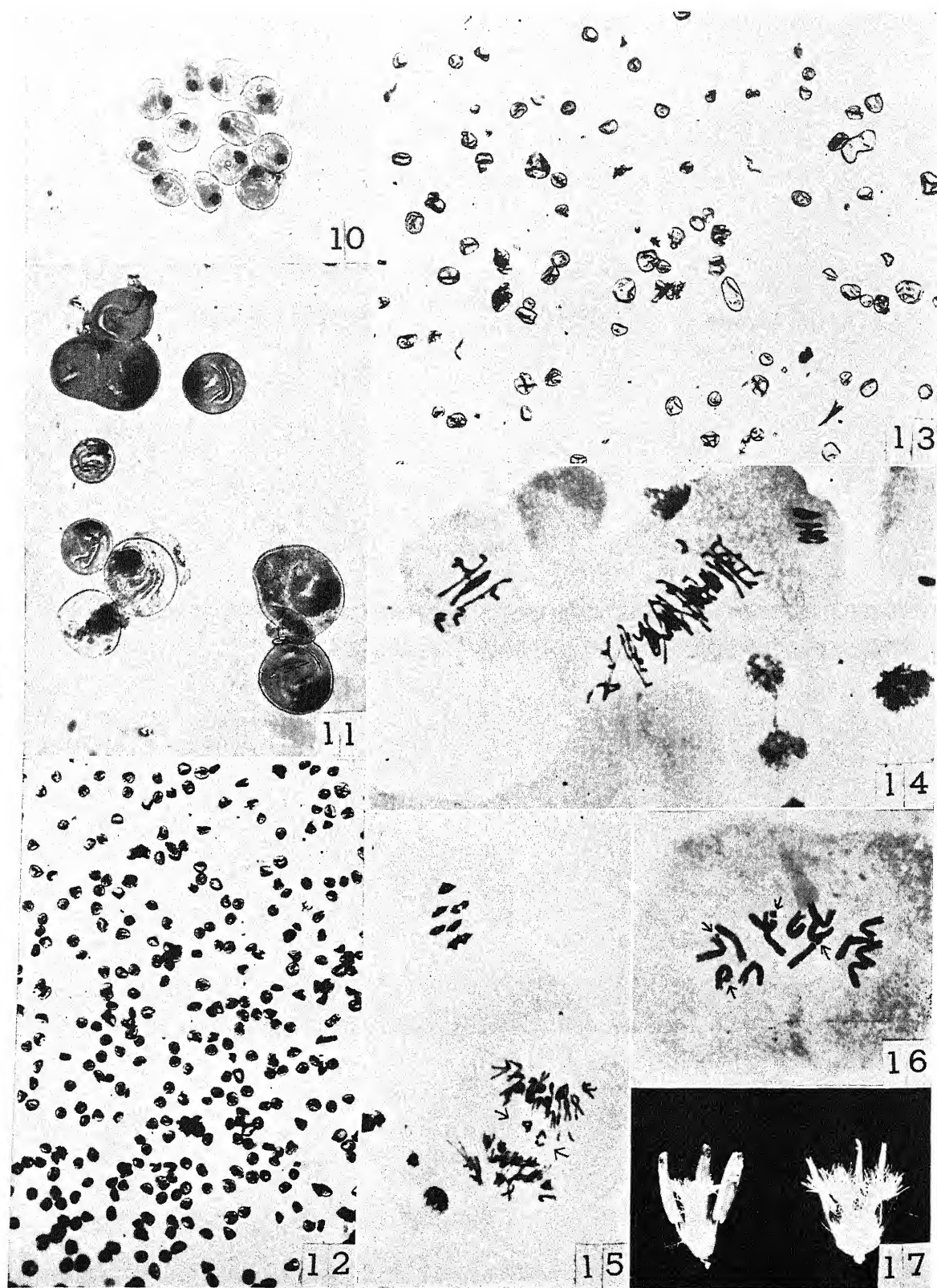
The second division usually proceeds with no more irregularities than might be expected from the number of chromosomes in the groups. In a few instances three second telophase nuclei were formed instead of four, indicating that cytokinesis following the first division was imperfect in these instances at least.

The microspores vary in size from much larger to smaller than normal. (Immature microspores of a normal plant and an *mu* plant are shown in fig. 10 and 11.) They are frequently irregular in shape, and two or more may appear to have united or to have been only partially separated by cytokinesis following the second meiotic division (fig. 11). In such fused spores with the contents continuous, the nuclei may be separate or united. Probably in the division of the cytoplasm of the large PMC masses, some portions contained no chromosomes. Some of these presumably remained attached to nucleated material. In the larger spores two or more nucleoli of various sizes are common, though one nucleolus is characteristic of normal barley microspores. The number and size of the nucleoli would, of course, depend on the number of nucleolar organizing, satellited chromosomes present and on how many nucleoli had fused.

The mature spores are wholly, or almost wholly, without starch (fig. 12, 13). It is apparent from these photomicrographs that the lateral florets (fig. 13) are more irregular than the central florets (fig. 12) at this stage as well as at meiosis.

A number of minor irregularities are observed in *mu* plants. (1) There is frequently a greater mixture or range of stages on a slide than is found in slides from normal plants. (2) At first metaphase in some cells the chromosomes are not condensed and shortened to the same degree as in normal plants. Therefore, the chromosomes appear long and tenuous. This condition appears in cells with only 7 pairs as well as in those with many (fig. 14). (3) Some chromosomes undergo equational division at first anaphase (fig. 15). (4) Univalents are occasionally present, and these may account for the equational divisions

Fig. 1-9.—Fig. 1. Diakinesis in a multiploid sporocytes (*mu*) plant of barley. No clear boundaries are present between the cells, and some groups of chromosomes (and nucleoli) are in proximity. $\times 390$.—Fig. 2. First meiotic metaphase in a normal plant of barley. There are seven bivalents. $\times 850$.—Fig. 3. A first meiotic metaphase group in an *mu* plant containing approximately 112 pairs of chromosomes. Groups with larger numbers have been observed. $\times 390$.—Fig. 4 and 5. Ribbons of pollen mother cell material in which groups of chromosomes are at first meiotic metaphase or anaphase. In figure 4 the groups are arranged along or across the ribbon of PMC material. It is also apparent in this figure that there is no striking difference between the timing of stages in the large and small groups. In both figures 4 and 5 it is clear that the spindle must be shorter in relation to the width than normal. Figure 4, $\times 390$; figure 5, $\times 190$.—Fig. 6. First meiotic metaphase in the same plant as figure 3. There are about 113 bivalents plus 3 quadrivalents (one near each end opened out into a ring, one zigzag at extreme right). $\times 680$.—Fig. 7. First metaphases in an *mu* plant showing how the chromosomes migrate (arrows) from one PMC to another or assemble along a common boundary between two cells. $\times 390$.—Fig. 8. First metaphase in an *mu* plant showing a group of 4 bivalents. $\times 850$.—Fig. 9. First meiotic telophase. The nuclei are elongate and near one end of the PMC material. $\times 390$.



mentioned in statement (3), or some chromosomes may undergo both equational and reductional divisions at the "first" division. Such "false univalents" were described by Darlington (1929) in hyacinths. (5) At first anaphase and later stages, few to many fragments are observed in some groups. These fragments and the tenuous chromosomes are not common in normal plants of barley but have been reported in a number of organisms and are probably due to unexplained physiological upsets.

Mitotic divisions in root tips and tapetal cells of *mu* plants appear normal (fig. 16) and all those counted had 14 chromosomes. Four of these fourteen chromosomes have satellites as in normal plants. The fact that *mu* individuals develop at about the same rate and attain almost the size of normal plants is further indication that mitosis is normal.

DESCRIPTION OF AFFECTED PLANTS.—At Aberdeen, Idaho, 1940, the progeny of a plant heterozygous for the "multiploid sporocytes" factor was measured for height. The average of 145 normal plants was 33.4 inches. The average for 37 *mu* plants was 31.1 inches. Affected plants were therefore somewhat reduced in height. Affected plants in this population, as in others, did not differ much in rate of growth or date of maturity. However, when the normal plants were near the hard dough stage, and were still green, the spikes of *mu* plants had turned a rich, golden yellow, so that affected plants could be distinguished by the color of the heads as well as by the absence of seeds. The yellow color of spikes on *mu* plants was brighter than the color of ripe spikes of normal plants and resembled the latter only superficially. The leaves at this stage were similar in color to those of normal plants. In another population 41 *mu* plants averaged 15 tillers while 16 *mu* sibs averaged 14 tillers per plant.

Anthers of *mu* plants apparently cease development shortly after meiosis. At flowering they are smaller and thinner than normal (fig. 17) and have a sickly, yellowish color.

"Multiploid sporocytes" plants rarely produce seed even when pollinated with pollen from normal plants. Though meiosis in anthers in some central florets seems to produce microspores with the normal complement of chromosomes, these anthers abort as do those in other florets of the head, and the spores are almost entirely lacking in starch. Megasporogenesis has not been studied though it is probably abnormal also since no seeds have been obtained by pollinating *mu* plants with pollen from normal individuals. This is not conclusive evidence, of course,

since other causes could prevent the development of seeds.

A few seeds have been found on affected individuals. Sixteen plants from such seeds found and grown by Harland Stevens at Aberdeen, Idaho, were examined by the writer. Of the 16, 7 were normal and 9 were *mu*. If the seeds had arisen from self-fertilization, all the plants would have been *mu mu*, whereas only 9 of the 16 were. This would indicate that the seeds probably arose from chance pollinations by nearby heterozygous sibs. Of the 7 plants which were not *mu mu* 4 were tested and found to be *Mu mu*. All 16 plants had 14 chromosomes. Even if the megaspore mother cells had produced ovules with 14 or more chromosomes, triploids, etc., might be rare or not occur, since it is a common observation that autotetraploids and parental diploids cross with difficulty if at all. The situations are not quite comparable, since the somatic tissues are all diploid (2n) in the case of the diploid plant with diploid (or polyploid) megaspores pollinated by pollen from a normal plant, and there are 4n and 2n somatic tissues in the respective individuals concerned in the tetraploid by diploid cross. However, the writer has studied a meiotic irregularity in einkorn wheat (*Triticum monococcum*) which results in a high proportion of diploid spores (microspores and apparently megaspores as well) and has observed no triploids but only diploids and unstable tetraploids among several hundred progeny. Certainly it appears that in this *T. monococcum* mutant there is selection against combinations of gametes which would result in triploids.

DISCUSSION.—The present paper adds another to the increasing number of observations on irregularities in the meiotic process and furthers the accumulation of evidence showing that meiosis is a delicately balanced series of changes which may be modified in devious ways at various stages. The mutant herein described furnishes an opportunity to observe meiosis under conditions of incomplete separation or fusion of pollen mother cells. Under these circumstances the bivalents at first meiotic metaphase are not widely dispersed in the microsporocyte material, but on the contrary, bivalents from many "cells" tend to congregate into a single metaphase plate. Such aggregation suggests more the effects of protoplasmic streaming than the effects of electromagnetic forces, since at metaphase the bivalents would presumably not be drawn together from considerable distances by magnetic

Fig. 10-17.—Fig. 10. Microspores from a normal barley plant shortly before the first mitotic division. $\times 190$.—Fig. 11. Microspores from an *mu* plant at about the same stage as figure 10. Note the irregular size and shape of the spores, large nuclei, etc. $\times 190$.—Fig. 12. Mature microspores of an *mu* plant from an anther of a central floret. Stained in iodine. Note the fairly uniform size of the grains, and the absence of starch. $\times 70$.—Fig. 13. From the same spike as figure 12 but from a lateral floret. Note the irregular size. $\times 70$.—Fig. 14. First meiotic metaphase in an *mu* plant showing long, tenuous chromosomes in a group of about 35 pairs, another with 7 pairs (left) and an adjoining group of 7 pairs of apparently normal length. (Upper right.) $\times 390$.—Fig. 15. First meiotic anaphase in a group of about 21 pairs showing chromatids of two chromosomes separating (arrows). Adjoining is a cell with seven bivalents at first metaphase. $\times 390$.—Fig. 16. A mitotic division in the root tip of an *mu* plant. It is apparently normal. Note the 4 satellites (arrows). $\times 850$.—Fig. 17. Mature sexual organs from a normal barley plant (left) and an *mu* plant (right). Note the difference in plumpness of the anthers.

attraction but would rather be kept apart, according to the conception of some cytologists.

This mutant also demonstrates that the spindle may be greatly increased in width without materially affecting its length, just as in certain other mutants and some hybrids the spindle has been observed to be much longer but little if any wider than normal. It was further apparent that the length of the spindle was largely independent of the size and shape of the pollen mother cell mass.

SUMMARY

A recessive factor in barley ($n=7$) was discovered which interferes with the normal development and behavior of a few to most of the microspores. As a result the number of bivalents in different metaphase plates varies from fewer than 7 to more than 100. In some groups quadrivalents are present, indicating that the chromosomes from dif-

ferent cells were in proximity at an early stage and were able to synapse, presumably because there was no barrier between them. It is also possible that cytokinesis was suppressed in some premeiotic divisions. In other groups, there is evidence that migration of the chromosomes took place in the formation of the metaphase plates. In large groups the spindle is many times wider than normal, but the length is not greatly changed. The microspores are variable in size and practically devoid of starch. Fertility is very low even when the florets were artificially pollinated with normal pollen. Mitosis is apparently regular, and affected plants are only slightly smaller than normal.

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EFFECTS OF MERCURY VAPOR UPON SEED GERMINATION ¹

Netta E. Gray and Harry J. Fuller

THE TOXIC effects of mercury, mercury compounds and mercury vapors upon animal organisms have long been known by animal physiologists, but relatively few investigators have studied the effects of these substances upon plants. The most searching experiments upon this subject have been those of Zimmerman and Crocker (1934), who studied the effects of mercury and of fourteen organic and inorganic mercury compounds upon the growth and development of actively growing angiospermous plants belonging to seventy-five genera. The plants were subjected to the mercury and mercury compounds in various ways: plants were enclosed within glass cases or placed under bell jars together with small receptacles containing metallic mercury or crystals of mercury compounds; plants were enclosed in similar cases within which were placed receptacles containing soil, sand, or tankage, to which metallic mercury or various mercury compounds had been added; plants growing under commercial greenhouse conditions were subjected to mercury vapors escaping from a single bench of rich soil containing mercuric chloride. These investigations indicated that growing plant parts, particularly flowers and leaves, are injured by exposure to mercury and mercury compounds. Plants of sixty-five genera proved to be dis-

tinctly susceptible to such injury, which was more pronounced in peach seedlings at higher temperatures (50°F. and above) than at lower temperatures (40°F. and 35°F.). The injury induced by the vapors from metallic mercury was very similar to that caused by vapors emanating from soils treated with mercuric chloride. This fact, coupled with the fact that vapor of metallic mercury was detected in the air above soil samples treated with the mercury compounds, is assumed to indicate reduction to the metallic state in the soil. Zimmerman and Crocker have reviewed briefly the few earlier papers on the subject, all of which treat of the effects of mercury or mercury compounds upon actively growing plant parts. Insofar as the authors of this paper have been able to determine, there have been no reports upon the effects of mercury vapor upon dormant seeds and young seedlings.

METHODS.—The experiments reported in this paper were arranged as follows:

Series I: Dry seeds of pea (Alaska), corn (Reid's yellow dent), bean (Burpee's Stringless Green Pod), radish (Scarlet Globe), sunflower, and cucumber (Early White Spine) were placed in open glass containers under a bell jar of approximately 15-liter volume, under which there was also included a 100 cc. beaker containing 50 cc. of metallic mercury. The

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TABLE 1 (Series I). Per cent germination of mercury-vapor-treated seeds at end of seven days.

Plant	Germinating pan		Sawdust flat		Sawdust pots	
	Hg-treated	Control	Hg-treated	Control	Hg-treated	Control
Peas	99	100	94	96	100	97.5
Corn	100	100	100	100	95	85
Radish	81	94	89	96
Beans	100	99	96	86	100	97.5

TABLE 2 (Series I). Average seedling length of mercury-vapor-treated seeds.

Plant	Time	Sawdust flat		Time	Sawdust pots	
		Hg-treated	Control		Hg-treated	Control
Peas	16 da.	32.5 cm.	31.6 cm.	16 da.	28.9 cm.	29.3 cm.
Corn	5 da.	5.5 cm.	5.3 cm.	14 da.	22.7 cm.	23.6 cm.
Beans	13 da.	32.3 cm.	30.6 cm.	13 da.	26.9 cm.	25.4 cm.
Radish	32 da.	8.2 cm.	8.5 cm.

bell jar was sealed to a ground-glass plate by means of petroleum jelly. The bell jar with the enclosed seeds and mercury was placed in a constant-temperature (21°C.) darkroom on April 1, 1940, and was allowed to remain there until October 1, 1940. At the end of this six-month period, 200 seeds of each species were placed in germinating pans and in moist sawdust in flats and in pots, and the percentage and rate of germination were determined; in addition, in some instances, measurements of seedling length and seedling dry weight were made to determine whether post-germination effects of the exposure to mercury were apparent. A control set of 200 seeds of each species was kept in another darkroom under similar conditions, but was not exposed to mercury vapor.

Series II: Seeds of pea, bean, radish, and corn were placed upon moist filter paper in Petri dishes, which were kept under bell jars, as described above for series I, together with a 100 cc. beaker containing 50 cc. of mercury. Determinations of the percentage and rate of germination of 100 seeds of each species were made at intervals, which varied for the different species used. Controls consisted of seeds germinated under similar conditions, except that they were not exposed to the mercury vapor.

Series III: Seeds of pea, bean, and corn were planted in moist sawdust in four-inch pots, which were placed in glass cases, approximately 4 feet x 3 feet x 3 feet. The cases were kept in a greenhouse,

the daily temperature of which fluctuated from 17° to 22°C. In each of three such cases, a Petri dish containing 15 cc. of mercury was placed beside the pots. In two of these cases one drop (.1 cc.) of mercury was thoroughly mixed with the sawdust before the pots were filled, in addition to the open dishes of mercury placed in the cases; in the third case, no mercury was added to the sawdust. Three similar cases were set up as controls. In two of these cases the sawdust was prepared with mercury as above and the third was left entirely without mercury.

RESULTS.—The results of these three series of experiments are presented in the following tables. In tables 1 and 2 are presented data from experiments of series I; in these tables only germination percentages and seedling lengths are recorded.

The data presented in tables 1 and 2 show clearly that there are no significant differences between control seeds and seeds subjected for six months to mercury vapor in the percentages of germination and in the early stages of seedling growth.

In table 3 are presented data from experiments in series I, showing the effects of mercury vapor upon the percentage of seed germination, seedling length, and seedling dry weight in six species, seeds of which were stored in the presence of mercury vapor for six months. In this experiment, the seeds were germinated in sawdust in flats, which were kept in a greenhouse room. Each of the figures in the

TABLE 3 (Series I).

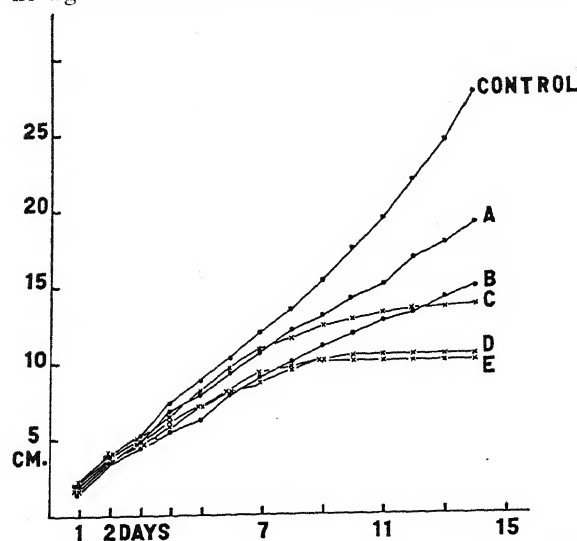
Plant	Per cent germination (after 6 days)		Seedling length (after 6 days)		Seedling dry weight (after 6 days)	
	Hg-treated	Control	Hg-treated	Control	Hg-treated	Control
Peas	89	91	5.1 cm.	5.4 cm.	.37 g.	.40 g.
Corn	96	93	4.8 cm.	4.6 cm.	.29 g.	.28 g.
Beans	98	99	8.8 cm.	8.4 cm.	.84 g.	.81 g.
Radish	86	83	6.9 cm.	6.7 cm.	.22 g.	.20 g.
Sunflower	81	84	5.8 cm.	6.0 cm.	.46 g.	.49 g.
Cucumber	84	83	7.6 cm.	7.9 cm.	.57 g.	.59 g.

TABLE 4 (Series II). Seed germination in mercury vapor.

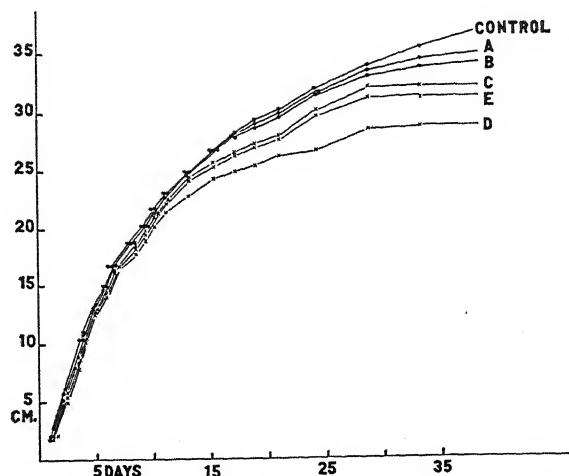
Plant	No. seeds		Per cent germination	
	Hg-treated	Control	Hg-treated	Control
Peas:				
4th day.....	210	210	16	7
5th day.....			71	65
6th day.....			98	98
Beans:				
3rd day.....	90	90	13	35
4th day.....			76	88
5th day.....			88	94
Radish:				
1st day.....	222	222	73	64
2nd day.....			86	82
3rd day.....			87	84
4th day.....			92	86
Corn:				
2nd day.....	90	90	30	60
3rd day.....			92	98
4th day.....			98	98
Sunflower:				
3rd day.....	150	150	40	58
4th day.....			60	75
5th day.....			68	88
6th day.....			90	91
Cucumber:				
5th day.....	150	150	45	56
6th day.....			53	73
7th day.....			80	85

columns on seedling length and seedling dry weight is an average of measurements of 100 seedlings.

The data presented in table 3 show that there are no significant differences between the control seeds



Graph 1. Pea seedlings grown in mercury vapor. A and B: Drop of mercury mixed with sawdust in each case. C: Dish of mercury set in the case. D and E: Drop of mercury mixed with sawdust and also an open dish of mercury in each case.

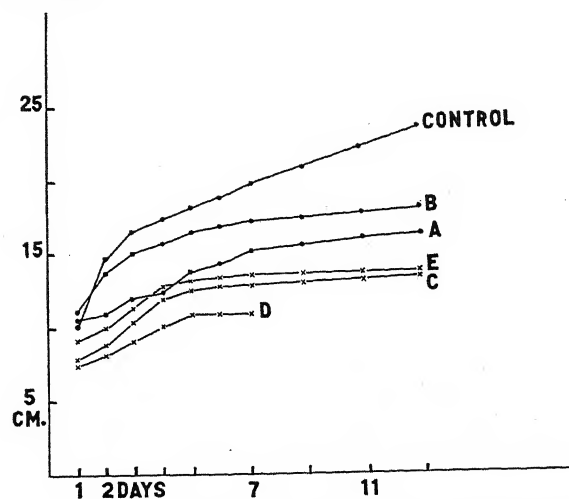


Graph 2. Corn seedlings grown in mercury vapor. A and B: Drop of mercury mixed with the sawdust in each case. C: Dish of mercury set in the case. D and E: Drop of mercury mixed with sawdust and also an open dish of mercury in each case.

and seeds exposed for six months to mercury vapor, with reference to percentage of germination, seedling length, and seedling dry weight, when the seeds were germinated and grown under greenhouse conditions.

In table 4 are presented data from series II, showing the percentage and rate of germination of seeds germinated in the presence of mercury vapor.

These data show that the mercury vapor present did not influence appreciably the percentage of seed germination, but that it did decrease somewhat the rate of germination in beans, corn, sunflower, and cucumber.



Graph 3. Bean seedlings grown in mercury vapor. A and B: Drop of mercury mixed with sawdust in each case. C: Dish of mercury set in the case. D and E: Drop of mercury mixed with sawdust and also an open dish of mercury in each case.

The results of series III are presented in graphs 1, 2, and 3, which show the growth in stem length in the various experimental cases. In these graphs, each curve represents the average of measurements on twenty seedlings. As the graphs show, the best growth of seedlings of peas, corn, and beans occurred in the controls, which contained no mercury. The growth of the seedlings grown without mercury in the substratum but with mercury in the Petri dishes in the glass cases was poorer than that of the controls. Poorest growth occurred in the seedlings grown in the cases in which mercury was present in the substratum as well as in the Petri dishes in the cases.

SUMMARY

Seeds of pea, corn, bean, radish, sunflower, and cucumber, stored for six months in nearly air-tight containers in the presence of mercury vapor suffered no ill effects as a result of that storage, for they showed no decrease in percentage of germination or in early seedling growth, as compared with seeds not exposed to mercury.

There was slightly delayed germination of seeds of these species when germinated in the presence of mercury vapor as compared with the germination of

seeds not germinated in mercury vapor. The final percentage of germination of the seeds grown in the presence of mercury vapor was not appreciably different, however, from the germination percentage of seeds not germinated in mercury vapor.

Seedlings of peas, corn, and beans grown in the presence of mercury vapor in the surrounding air and mercury in the substratum grew more poorly than seedlings grown with mercury vapor present only in the air. Seedlings under both these conditions grew more slowly than seedlings completely removed from mercury and, in addition to their stunted growth, showed other signs of injury: yellowing of leaves, failure of leaf development, and early leaf fall.

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THE EFFECT OF VITAMINS ON TEN STRAINS OF SACCHAROMYCES CEREVISIAE¹

Leon H. Leonian and Virgil Greene Lilly

Bios is a collective term; it includes a number of growth substances required by different strains of yeasts. Five of its members are now available as chemically pure compounds; they are thiamin, pyridoxin, inositol, pantothenic acid, and biotin. Additional factors, known to exist in liver, in yeast extract, and in other organic substances, are yet to be isolated in pure form. While these may induce significant increases, they do not constitute critical factors in growth; therefore, it is now possible to conduct fundamental studies in the nutrition of yeasts without resorting to various "concentrates" which, in the absence of the pure substance served as growth factors. The work of Williams, Eakin and Snell (1940) is the only important one on the interaction of the foregoing five substances. For a comprehensive survey of the more pertinent literature on this subject the reader is referred to Williams (1941).

In the following experiments, the writers used ten strains of *Saccharomyces cerevisiae* and all possible combinations of the five growth factors.

MATERIALS AND METHODS.—The following is a list of cultures of *Saccharomyces cerevisiae* used in this work:

1. "Old Process," secured from American Type Culture collection.

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2. "Gebrüder Mayer," secured from American Type Culture collection.
3. "Fleischmann," isolated from a cake of yeast.
4. "Red Star," isolated from a cake of yeast.
5. ATCC2331, received from National Regional Research Lab., Peoria, Ill.
6. "Botton Yeast," originally from Kluyver, Delft, Holland. Obtained from the Department of Agricultural Bacteriology, University of Wisconsin.
7. "Top Yeast," same origin as No. 6.
8. National Grain Yeast Corporation yeast No. 1, received from Regional Laboratory, Peoria, Ill.
9. National Grain Yeast Corporation, yeast No. 2, same origin as No. 8.
10. Originally from Pasteur Institute, Paris; this has been used extensively in growth factor studies; obtained from the Regional Laboratory, Peoria.

The following nutrient solution was used as standard medium:

Dextrose	25	gm.
l-aspartic acid	1.50	gm.
(NH) ₄ SO ₄	0.75	gm.
KH ₂ PO ₄	1.0	gm.
MgSO ₄ ·7H ₂ O	0.50	gm.
Na ₂ CO ₃	0.60	gm.
Tl ₂ SO ₄	0.8	mg.
ZnSO ₄ ·7H ₂ O	1.0	mg.
MnSO ₄ ·4H ₂ O	1.77	mg.
H ₃ BO ₃	1.0	mg.
Fe(NO ₃) ₃ ·9H ₂ O	1.5	mg.
CuSO ₄ ·5H ₂ O	0.1	mg.

KI	0.1 mg.
Thiamin chloride ²	1 part in 20 million
Pyridoxin hydrochloride	1 part in 20 million
Calcium pantothenate	1 part in 20 million
i-inositol	5 parts per million
Biotin methyl ester	1 part in 2 billion
Distilled water	1,000 ml.

This solution was adjusted to pH 5.0 before sterilization. When used for stock cultures, 0.02 per cent yeast extract (Difco) was added to insure a more vigorous growth. The vigor of the stock cultures and that of the inoculum, which is removed from such cultures and transferred to the test solution, has an important bearing on the final results. If a given inoculum is not taken from a culture of optimum vigor, its behavior often becomes erratic and the results unreliable. All stock cultures for inoculum were carried on a liquid medium with 25 ml. of the nutrient solution per 250 ml. flask. After incubating at 25°C. for three days the cultures were ready to furnish inoculum. This consisted of one standardized loopful of the cell suspension for each flask to be inoculated. The platinum wire was B & S 24-gauge, double loop, with an inside diameter of 2.5 mm. Such a loop removes approximately 0.01 mg. of cells (calculated on the dry basis) from the stock solution. The culture flasks were arranged on a motor-driven shaker which was timed to agitate the cultures for ten minutes every hour. After incubating for various lengths of time, the yeast cells of each flask were filtered separately through alundum crucibles RA-360 under a vacuum of 650 mm. Hg. The cells were then washed three times with distilled water, dried at 85°C. and weighed. After that, the crucibles were washed, heated in a muffle furnace at 800°C., cooled, washed with three changes of distilled water, dried at 85°C. and tared. This was repeated after each harvest. When less than 5 mg., the determinations were made with the aid of an electrophotometer.³

Every experiment reported in this paper was repeated at least three times, some as many as ten times. The cultures were always made in duplicate. Thus, each figure in the following tables represents the average of at least six, and often as many as twenty determinations. As a general rule, the results were fairly close and reproducible, but whenever the nutritional deficiencies were too acute, the behavior of the yeasts was more erratic. This necessitated a great number of determinations before a satisfactory average could be established.

Strict bacteriological methods were employed throughout the work, and all glassware was thoroughly cleaned before using. Cotton used for plug-

²Thiamin chloride, pyridoxin hydrochloride, and calcium pantothenate were obtained from Merck, biotin methyl ester from SMACO, and inositol from Pfanstiel.

³The writers have observed considerable discrepancy between electrophotometer readings and actual weights. The same weight of different strains of yeasts may give different readings on the machine. Furthermore, the same weight of a given strain of yeast grown under different conditions, may give readings varying as much as 200 per cent.

ging was first placed in a large Barnstead Soxhlet extractor and extracted with 90 per cent alcohol for twenty-four hours. This eliminated or critically reduced the growth factors present in the cotton.

Some investigators practice a thorough washing of the cells before using them for inoculum. This, so far as our own conditions were concerned, was unnecessary. After three days' growth the yeast did not leave any appreciable amount of growth factors in the medium. This was demonstrated by filtering three-day-old cultures, adding salts, aspartic acid, and dextrose, adjusting the pH, sterilizing, and inoculating with yeasts; no appreciable growth followed. But when the necessary vitamins were added to this medium a good growth resulted. Furthermore, one loopful of the standard solution containing all the vitamins, when added to a flask of medium containing no vitamins, failed to produce any growth; nor did five or ten loopfuls; twenty loopfuls induced very faint growth, about 0.2 mg. per flask.

TIME, TEMPERATURE, QUANTITY-OF-INOCULUM FACTORS.—The filamentous fungi, heterotrophic with respect to vitamins, cannot make any appreciable growth without the presence of an exogenous supply of these substances, regardless of time. Such is not the case with the strains of yeast used in this work. If given enough time, they will make some growth; often this growth is considerable. Therefore, in studies on the nutrition of yeasts, it is imperative to establish a time standard for obtaining an optimum growth. Since yeasts make a more rapid growth at comparatively high temperatures, time and temperature standards must be established simultaneously. There is still a third important factor, namely, the quantity of inoculum. Where a quantity of inoculum weighing no more than 0.01 mg. may induce no growth within a certain period, another inoculum of the same yeast weighing 0.2 mg. may produce a fairly good growth. At best, however, any quantity-of-inoculum factor must be an arbitrary one. The writers have selected 0.01 mg. (estimated on the basis of dry weight) because it was found to be very convenient and can be easily duplicated.

One may establish a particular time-temperature-quantity-of-inoculum standard and find it still inadequate because some yeasts require a comparatively short period to attain their optimum growth when there is no critical nutritional condition, such as exists in the absence of one or more growth factors from the medium; when such a condition exists, a longer period may be required. Consequently, the time factor cannot be uniformly standardized. Williams *et al.* (1940) believe that twenty-four hours at 30°C. is the most interesting and practical length of time, and that longer periods are chiefly of academic interest. The important factor to consider is whether or not a given length of time will enable the growth of a given yeast to attain a dependable equilibrium despite deficiencies in the medium. The writers have found that at 25°C. reproducible results could not be obtained within twenty-four and, in many cases, within forty-eight hours. However,

after seventy-two hours a greater uniformity was observed among the results of different replications of a given experiment. Consequently, seventy-two hours at 25°C. has been established as the most satisfactory time-temperature standard. Nevertheless, the more pertinent results obtained with a shorter period have also been recorded (table 2).

EXPERIMENTS.—Only thirteen combinations of vitamins (out of the possible thirty-one) induced any growth; all others failed. After seventy-two hours' incubation, there was no growth in solutions containing no biotin. Only yeast no. 10 occasionally made a slight growth; oftener it failed to grow. Perhaps it should be stated that by "no growth" we do not wish to convey the idea that the inoculum did not increase to some extent; we merely mean to state that to the eye the medium remained clear. There was no growth when biotin and one or more other growth factors were left out; nor did any growth occur when thiamin, pyridoxin, inositol, pantothenic acid, and biotin were added singly to the medium. Williams *et al.* (1940) report that Gebrüder Mayer, Old Process and Fleischmann yeasts made considerable growth in the presence of pantothenic acid alone, and that the last-mentioned yeast made a good growth in the medium to which only biotin was added. Results obtained under our conditions fail to support this assertion of Williams *et al.* The following combinations also failed to support any growth in any of the yeasts: thiamin and pyridoxin, thiamin and pantothenic acid, thiamin and inositol, thiamin and biotin, pyridoxin and pantothenic acid, pyridoxin and inositol, and pyridoxin and biotin. There was no growth in the control. This is contrary to the results

of Williams *et al.*, who reported discernible growth in their blanks at the end of three days.

Table 1 gives the results obtained after seventy-two hours at 25°C. Each figure indicates the average growth per flask of 25 ml. solution. The yields that are less than 1 milligram per flask should not be regarded as significant; for all practical purposes they may be considered as showing no growth.

Table 1 shows that with the exception of yeasts no. 8 and 9 all others produced uniformly high yields when grown in the standard solution containing all five growth factors. When, however, 0.02 per cent yeast extract was added to this medium, the yield of no. 8 was 71 mg. as compared with 40 in the standard solution, and that of no. 9 was 73.1 instead of 45.5. The yield of all yeasts except that of no. 5 was from 70 to 74 mg.; no. 5 produced 66.3 mg.

When thiamin alone was left out, the Old Process yeast was the only one affected; its yield dropped from 54.1 to 10.7 mg. When pyridoxin was left out, no significant changes in the yield of any yeast was observed; but when both thiamin and pyridoxin were omitted, the yield of Old Process further decreased from 10.7 to 3.2 mg., and yeasts no. 5 and no. 9 sharply reduced their yield to 14.9 and 11.4 mg., respectively. This shows that both thiamin and pyridoxin play an essential role in the growth of these yeasts, and that, while an organism may be able to synthesize thiamin in the presence of pyridoxin or synthesize the latter in the presence of thiamin, it cannot effectively synthesize both vitamins at the same time, at least not within a period of three days.

TABLE 1. The effect of growth factors on yeast as expressed by the weight in mg. of dry cells per flask after incubation of seventy-two hours at 25°C.

Treatment	Yeast 1	Yeast 2	Yeast 3	Yeast 4	Yeast 5	Yeast 6	Yeast 7	Yeast 8	Yeast 9	Yeast 10
All five factors present	54.1	62.0	50.0	56.3	52.2	58.6	52.9	40.0	45.5	54.9
Minus thiamin	10.7	60.6	50.3	53.3	51.1	58.1	54.6	40.2	45.3	56.7
Minus pyridoxine	57.4	58.8	50.2	55.9	49.3	61.9	52.1	39.8	44.3	54.0
Minus inositol	7.2	13.8	50.2	56.2	55.7	64.6	17.1	43.6	4.8	57.1
Minus pantothenic acid ...	8.7	3.0	0.5	3.5	1.4	20.2	0.6	0.5	0.5	5.0
Minus biotin	0.4	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.6
Minus thiamin and pyridoxine	3.2	59.2	50.0	54.0	14.9	55.9	60.1	41.9	11.4	52.5
Minus thiamin and inositol.	0.4	8.6	53.6	40.0	59.1	53.5	16.9	37.1	0.9	55.2
Minus thiamin and pantothenic acid	6.4	2.5	0.5	4.2	15.6	51.3	0.4	0.3	0.4	7.3
Minus pyridoxine and inositol	12.1	19.3	51.4	44.4	51.1	61.6	8.1	41.9	2.5	53.5
Minus pyridoxine and pantothenic acid	4.3	0.5	0.5	0.4	6.8	23.1	0.3	2.5	0.3	5.7
Minus pantothenic acid and inositol	2.4	1.3	0.3	1.6	5.1	25.4	0.3	0.2	0.2	6.7
Minus thiamin, pyridoxine, pantothenic acid	2.5	2.7	0.6	4.4	5.9	52.4	0.3	0.3	0.7	6.4
Minus thiamin, pyridoxine, inositol	2.4	7.7	47.9	43.5	7.2	54.3	13.3	29.8	0.5	53.6
All five factors plus 0.02% yeast extract	71.2	74.8	72.7	74.8	66.3	70.5	73.6	71.0	73.1	71.2
Control: No growth substances added	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4

Only four yeasts, no. 1, 2, 7 and 9, manifested a sharp reduction in yield when inositol alone was left out of the medium; when inositol and thiamin both were left out, the yield of yeast no. 1, 2 and 9 was further reduced, and that of no. 7 remained the same. Yeast no. 4 which was unaffected when either thiamin or inositol alone was left out, reduced its yield from 56 to 40 mg. when both of them were omitted. In the absence of pyridoxin and inositol, yeast no. 2 did not show any further reduction; in fact, it did slightly better. Yeast no. 7, on the other hand, was reduced from 17.1 mg. when inositol alone was left out, to 8.1 when both inositol and pyridoxin were omitted. Yeast no. 9 also manifested a reduction. However, when yields become very small, the differences may seem very large on the percentage basis, and insignificant on the basis of actual yield produced.

In the absence of pyridoxin and pantothenic acid, or inositol and pantothenic acid, the yield of all ten yeasts was sharply reduced. This was to be expected because the omission of pantothenic acid alone brings about a sharp reduction.

Yeast no. 6 made a better growth in the absence than in the presence of inositol. When only inositol or only biotin was added to the medium, no growth ensued, but in the presence of inositol and biotin together this yeast made almost as good growth as it did in the presence of all five growth factors. Thus, the addition of an apparently unnecessary substance to biotin made the difference between growth and no growth. Similarly, pantothenic acid alone failed to induce any growth in this yeast; added to biotin, it helped to produce an excellent growth. When supplied with biotin, and either pantothenic acid or inositol, yeast 6 synthesized the missing factor. Another significant fact to be noted is that in the presence of all five vitamins this yeast produced 58.6 mg. of cells; when thiamin was omitted, the yield remained the same; when pyridoxin and pantothenic acid were omitted, the yield was reduced to 23.1 mg., but when thiamin, pyridoxin, and pantothenic acid were left out, the yield was 52.4 mg. Apparently thiamin is toxic in the absence of pyridoxin and pantothenic acid.

Yeast 10 attained its optimum growth when pantothenic acid and biotin alone were added to the medium; yeasts 3 and 4 showed a slight decrease, while the yield of no. 8 was reduced from 40 to 29.8 milligrams.

THE EFFECT OF HEAVY INOCULUM.—The amount of inoculum was increased from 0.01 mg. to 0.2 mg., and all the work reported in table 1 was repeated. Three-day-old cultures were allowed to stand for three hours to allow the yeast to settle. The clear solution was then poured off, leaving behind nearly all of the cells and 1 ml. of the solution. One loopful of this heavy suspension, estimated to contain twenty times the amount of cells in the light inoculum, was transferred to each flask. Only a few of the more pertinent results are given here. In the absence of thiamin, the Old Process yeast failed to show any

increase in the yield when the inoculum was twenty times the normal amount. However, in the absence of inositol its yield was doubled by the heavy inoculum; that of Gebrüder Mayer increased from 13 to 35 mg., while yeast no. 9 increased from 4.8 to 28 mg. When pantothenic acid was omitted, the yield of yeast no. 4 went up from 3.5 to as much as 50 mg. However, the growth was not always as much as this, but sometimes varied down to 16 mg. When pantothenic acid was omitted, yeasts 3, 5, 7, 8 and 9 failed to make any appreciable growth either with light or with heavy inoculum. These five yeasts manifested a similar indifference to heavy inoculum when both pantothenic acid and inositol, pyridoxin and pantothenic acid, and thiamin and pantothenic acid were omitted. When all other growth factors were present except biotin, the heavy inoculum did not induce more than 1 to 4 mg. of cells in all cases except yeast no. 10, where the yield varied from 20 to 40 mg.

Why in so many cases did a heavy inoculum induce good growth while a light inoculum failed? The first answer that comes to mind is that the larger numbers of cells carry enough of the missing vitamin into the deficient medium to induce growth. However, this explanation does not rest on a sound basis as shown by the results of the following experiment: One loopful each of a heavy suspension of cells (0.2 mg.) of yeast no. 4 was transferred to a number of flasks containing 25 ml. of the nutrient solution and all the growth factors except pantothenic acid. Some of the flasks were boiled over an open flame for five minutes to kill the yeast cells and to extract their vitamin content; the solution was then cooled and reinoculated. The remaining flasks were not boiled. After an incubation period of seventy-two hours the cells were harvested. The boiled solutions yielded only 3 mg. per flask while the unboiled ones yielded 30 to 45 mg. This shows that the pantothenic acid content of 0.2 mg. yeast cells was too small to have any effect, and that the factor responsible for the increased growth was associated with the living protoplasm. It may be argued that some thermolabile substance present in the cells stimulated the living protoplasm into synthesizing vitamins. However, this does not seem to be the case because various amounts of acetone extracts and chloroform dialyzate, obtained without heat and aseptically, failed to induce increased growth. Some may reason that each living cell of the inoculum, being unable to multiply extensively in a deficient solution, will cease to grow after exhausting its reserve supply of vitamins; consequently, the larger the inoculum, the larger will be the yield. This explanation would deserve consideration if the differences between the results of the light and the heavy inoculum were no more than a few milligrams; but we have seen that often a heavy inoculum in a deficient solution may bring about an increase of several hundred per cent. We know that one loopful of a heavy suspension of yeast cells contains such an infinitesimal amount of pantothenic acid as to be entirely ineffective in a medium lacking this vitamin. Our experiments have shown

that 1 part of pantothenic acid in 250 million parts of the medium induces but 4 or 5 mg. of cells of yeast no. 4; this is not much more than the growth made in the total absence of pantothenic acid. In another experiment the amount of inoculum per flask was increased to 30 mg. This much inoculum was placed in a flask containing 25 ml. of the nutrient solution but with none of the vitamins. Some of the flasks thus prepared were boiled and re-inoculated without filtering off the insoluble parts of cells; others were left unboiled. The latter yielded a total of 52 mg. of cells per flask and the former only 32 mg. After deducting from this 32 mg. the weight of the insoluble parts which resulted from the boiling of 30 mg. of inoculum, there remained only 18 mg. of cells per flask of boiled solution. In other words, 30 mg. of the yeast cells contained enough vitamins to produce 18 mg. of new cells. Thus, a heavy inoculum consisting of 0.2 mg. of cells contains enough vitamins to induce a yield slightly more than 0.1 mg. of new cells per flask, a very insignificant growth. According to these results, therefore, the carrying-over phenomenon may be considered as being of no consequence whatever.

The writers offer the phenomenon of dissociation, and probably that of segregation, in explanation of the effect of heavy inoculum. It is a well-known fact that upon longer incubation, yeasts can make considerable growth in the absence of a given vitamin, whereas only poor or no growth will result when the incubation period is reduced. This is not a matter of slow synthesis of vitamins on the part of the cells; rather, it is a selective process whereby restricted numbers of cells with autotrophic tendencies, scattered in an overwhelming population of heterotrophic cells, find the opportunity to multiply and to establish themselves in a solution deficient with respect to a given vitamin. The heavy inoculum used by the writers contained twenty times more cells than the light inoculum, and, therefore, twenty times more chances of containing cells with autotrophic

tendencies. The principal difference between the light and the heavy inoculum is that the large numbers of cells of the latter enable the yeast to accomplish within a shorter period the same thing that a light inoculum does after a longer incubation. In addition, there is the chance factor; if a loopful of heavy inoculum contains the minimum number of cells with autotrophic tendencies, a good growth will follow; but if the inoculum happens to contain fewer than the minimum numbers the growth will be poorer. This chance factor may be responsible for most of the erratic results observed in some of the more critically deficient media, as well as for the fact that under identical conditions the heavy inocula sometimes induce good growth and sometimes poor growth. The greater the vitamin deficiency, the greater will be the erratic behavior. But once the autotrophic tendency has been developed by several passages in a solution from which one or more vitamins are omitted, yeasts grow as readily in deficient solutions as they do in the complete medium.

THE EFFECT OF A SHORTER PERIOD OF INCUBATION.—As the time of incubation was reduced, fluctuations in growth became so great and so frequent as to be utterly unpredictable. At one time, the same organisms, under the same conditions, grew remarkably well, and, at another time, poor or no growth resulted. Even in duplicate cultures one flask often grew well, the other very poorly. Such inconstant results were almost always linked with the absence of one or more growth factors. For instance, in the complete solution containing all growth factors, major fluctuations were extremely rare; when, however, pyridoxin was omitted, some fluctuations began to appear, but they reached their peak when pantothenic acid was omitted or when inositol and thiamin were left out. The increase in time of incubation tended to equalize sharp differences, and we can state that most of the results in table 1 are fairly reproducible; however, the same cannot be said about the results shown in table 2; the different replica-

TABLE 2. *The effect of growth factors on yeast as expressed by the weight in mg. of dry cells per flask after incubation of forty-eight hours at 25°C.*

Treatment	Yeast 1	Yeast 2	Yeast 3	Yeast 4	Yeast 5	Yeast 6	Yeast 7	Yeast 8	Yeast 9	Yeast 10
All five factors present	52.8	61.8	56.1	52.9	50.1	57.3	49.1	50.6	44.7	42.7
Minus thiamin	2.8	60.0	50.4	50.3	50.9	28.1	20.5	30.0	49.5	57.1
					(29.2)			(17.2)	(11.6)	(17.8)
Minus pyridoxine	53.2	58.4	46.5	49.5	44.5	24.1	39.9	38.9	38.1	41.5
			(15.7)	(19.7)		(51.0)		(17.0)		(27.5) (6.0)
Minus inositol	3.0	4.4	25.1	54.2	52.9	49.8	3.0	38.0	4.2	47.0
				(39.6)	(20.2)	(14.4)		(10.1)		(10.1)
Minus thiamin and pyridoxine..	3.4	59.2	52.4	52.2	3.2	20.4	42.9	44.2	5.8	29.8
			(37.6)	(23.5)		(43.4)	(11.8)	(5.9)		(7.7)
Minus thiamin and inositol.....	0	5.6	14.9	21.6	15.2	8.7	5.0	9.2	0	44.2
			(6.3)		(41.6)	(3.6)		(0)		(9.4)
Minus pyridoxine and inositol..	0	7.6	10.3	16.6	11.4	11.1	0.4	0	0	0
	(4.5)	(0)	(27.7)	(31.8)	(29.6)	(36.7)		(7.9)	(4.0)	(9.2)
Minus pantothenic acid and bio- tin	0	3.3	14.5	39.8	3.1	11.0	0.6	3.9	0	10.4
		(0)	(27.3)	(12.1)	(0)	(1.0)		(13.5)		(32.0)

tions gave such wide variations in the results as to render any sort of statistical treatment impossible. The writers found it necessary to give two averages for each treatment; one, the average of high yields, and the other that of the low yields. The less frequently appearing yields are given in parenthesis.

Table 2 shows that often the time factor determines whether or not certain exogenous substances are needed by various strains of yeasts. For instance, after seventy-two hours' incubation, the Old Process yeast was the only one that failed to grow well in the absence of thiamin; but when this period was reduced to forty-eight hours, yeasts no. 6, 7, and 8 also failed to grow well, while yeasts no. 5, 8, 9, and 10 became erratic in their behavior, sometimes growing as well as ever and sometimes being markedly reduced in growth. At seventy-two hours none of the yeasts was affected by the omission of pyridoxin, while at forty-eight hours, a number of them were restricted. Similar effects are to be seen throughout table 2.

At forty-eight hours, and in the absence of certain growth factors, many yeasts did not attain their optimum growth. Whether their erratic behavior was caused by normal fluctuations or by reversible dissociation is difficult to determine. In a later publication, the writers will show that autotrophic habits for a number of growth substances can be built up in all the strains of heterotrophic yeasts listed in this paper, so that an organism that was formerly dependent on thiamin, inositol, or pantothenic acid can now grow just as well without an exogenous supply of these substances. Since yeasts seem to possess this autotrophic habit, even though it may be masked by strong heterotrophic tendencies, it is only natural that this habit should emerge every now and then and cause the inconsistent behavior noted in table 2. Therefore, so far as conditions outlined in this paper are concerned, an incubation period of seventy-two hours is the best. While it does not eliminate fluctuations and inconsistencies alto-

gether, it reduces them to a minimum so that reasonably reproducible results may be expected.

SUMMARY

The effect of thiamin, pyridoxin, inositol, pantothenic acid, and biotin, singly and in all possible combinations, was tested on ten strains of *Saccharomyces cerevisiae*. After seventy-two hours' incubation at 25°C. only one organism was found to be dependent on thiamin, four were dependent on inositol, all ten yeasts were greatly restricted in growth when pantothenic acid was omitted, and no growth followed when biotin alone was left out. None of the yeasts were affected by lack of pyridoxin, but when thiamin and pyridoxin or thiamin and inositol were omitted, some yeasts were reduced in growth, although they were unaffected when these substances were left out one at a time. Some yeasts were unable to produce optimum growth when only two growth factors were present; some required three and some four. When the incubation period was reduced to forty-eight hours, a number of the yeasts manifested dependence on one or more growth factors which did not seem necessary when the incubation period was extended to seventy-two hours. Often the quantity of inoculum controlled growth. When 0.01 mg. of cells constituted the amount of inoculum, very poor growth was made by some yeasts; when this was increased twenty times, very good growth followed. However, in some other cases, the quantity of inoculum was without any apparent effect.

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ANATOMY OF THE INFERIOR OVARY OF DARBYA¹

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THE STRUCTURE of the inferior ovary has been a much disputed question since the time of Linnaeus. A final solution of this problem would be important not only to the anatomist in interpreting the vascular structure of the flower but also to the taxonomist in determining phylogenetic relationships in the

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flowering plants. There are two interpretations of the inferior ovary that have been widely accepted. The first is that it is entirely floral in nature and consists of the sepals, petals and stamens fused with each other and with the ovary. The second interpretation is that the ovary is embedded in tissues of the receptacle. A third possibility that has been suggested is that the tissues surrounding the ovary may be axial in the lower portions and floral above.

The first of these theories assumes that the inferior ovary originated by the progressive fusion, phylogenetically, of the sepals, petals and stamens to each other and then to the ovary. This theory is supported by numerous investigators with anatomical evidence which is most complete and convincing.

This evidence is based on the assumption that a flower is a determinate stem bearing appendages which correspond to leaves, at least in so far as vascular traces are concerned (Eames, 1931). Evolutionary series have been demonstrated for various plant groups which indicate that adnation, or fusion of floral parts of separate whorls, results in an inferior ovary. One of the most complete of these series has been established by the work of Bonne (1928), Jackson (1934) and MacDaniels (1940). This series indicates that the floral tube or disc in most of the Rosaceae originated by fusion, first of the sepals, petals and stamens, then of the vascular bundles supplying these structures. All of the evidence thus far indicates that the inferior ovary of the Rosaceae resulted from a progressively closer union between a floral tube of this type and the ovary. On the basis of this and other series it seems evident that the inferior ovary in the Rosaceae, Ericaceae (Eames, 1931), and in various other families, is composed only of floral tissues. Another type of evidence has been used by Saunders (1925) in interpreting the structure of the ovaries in the Iridaceae and Begoniaceae. On the basis of the leafskin theory, she concluded that in these families the inferior ovary is appendicular.

The evidence for the axial or receptacular interpretation of the inferior ovary has, from the time of Payer (1857), been presented primarily by the developmental morphologist. The ontogeny of the apple flower as described by Kraus (1913) serves to illustrate this type of evidence. The various floral parts first appear as separate protuberances on the growing point. Then a zonal meristem develops behind or below these protuberances so that the ovary becomes enclosed and the developing floral parts are lifted up together above the ovary. This method of development is interpreted as an indication that the tissues formed by the zonal meristem are axial or receptacular. Adherents of the axial theory have used other types of evidence, such as abnormal flowers and gross structure of fruits, to support the theory, but as yet no really convincing anatomical evidence has been presented. For this reason the theory, though very widely accepted for the past fifty years, has been rejected by most anatomists of recent times in favor of the appendicular theory. The extreme of the axial theory is illustrated by the work of Bugnon (1926), who concluded that in the Begoniaceae the wall of the inferior ovary is composed entirely of receptacular tissues.

Bonne (1928) and Jackson (1934) concluded independently that in the genus *Rosa*, and in several other genera of the Rosaceae having floral cups, the lower portion of the floral tube is axial while the upper portion is floral in nature. Their conclusions are based primarily on the presence of recurrent traces in the lower portion of the cup. These traces are inverted, that is the xylem is located outside the phloem, and they give rise to traces to the carpels that are attached at the base of the floral tube. Thus the tube is considered to be receptacular up to ap-

proximately the point of origin of the recurrent traces, and the anatomical tip of the axis is the lowest point in the cup. Approximately the same type of structure has been described for *Calycanthus* by Smith (1928), except that the situation here is complicated by the presence of cortical bundles. These three workers have all considered the cup-shaped structure described to have arisen by invagination of the growing point. Smith (1928), Bancroft (1935) and MacDaniels (1940) have suggested the possibility that an inferior ovary that is receptacular, at least in the lower regions, might be derived by fusion of such a cup to the ovary.

Many additional papers dealing with the various interpretations of the inferior ovary have already been adequately reviewed by Bancroft (1935), by Wilson and Just (1939) and by others, so that a more complete discussion is not necessary here.

The floral anatomy of species belonging to the Santalaceae has received little attention. There are descriptions of the floral anatomy of only three genera and these descriptions are so much at variance with each other that no comparisons can be made. Since the floral anatomy of these and other genera will be described in a later publication, these papers are not discussed in detail here. Van Tieghem (1869), from a study of *Thesium* and *Osyris*, concluded that the inferior ovary in the Santalaceae is appendicular. He considered the calyx to be fused with the tricarpellary ovary, though both structures remain vascularly independent. He also concluded that the placental stalk is composed entirely of carpellary tissue and that the pedicel does not extend beyond the point of insertion of the carpel traces. Dowding (1931) described but did not illustrate the floral anatomy of two species of *Comandra* and interpreted the tissues surrounding the ovary as appendicular. As an adherent of the theory of carpel polymorphism he concluded that the ovary consisted of an outer whorl of five sterile carpels and an inner whorl of five fertile carpels, the latter of which forms the placental stalk. Schaeppi and Steindl (1937) described the anatomy of both male and female flowers of *Osyris alba*. Numerous bundles in a ring enter the floral tube of the male flower. All of these disappear near the top of the tube, except those opposite the sepals which represent combined sepal-stamen traces and separate to supply these structures. In the female flower there is an outer ring of many bundles which are interpreted as bundles of the receptacle or "Achsenmantel" and an inner ring of numerous traces interpreted as bundles of the pistil. Apparently the principal reason for considering the inner traces to belong to the ovary is that they are not present in the male flowers. In addition to these traces there are three dorsal carpellary bundles located inside the two rings of bundles and a single trace entering the placental column. It was suggested that the placental stalk may have a core of axial tissue.

The placental stalk in the Santalaceae has been compared with the free-central placenta of the Pri-

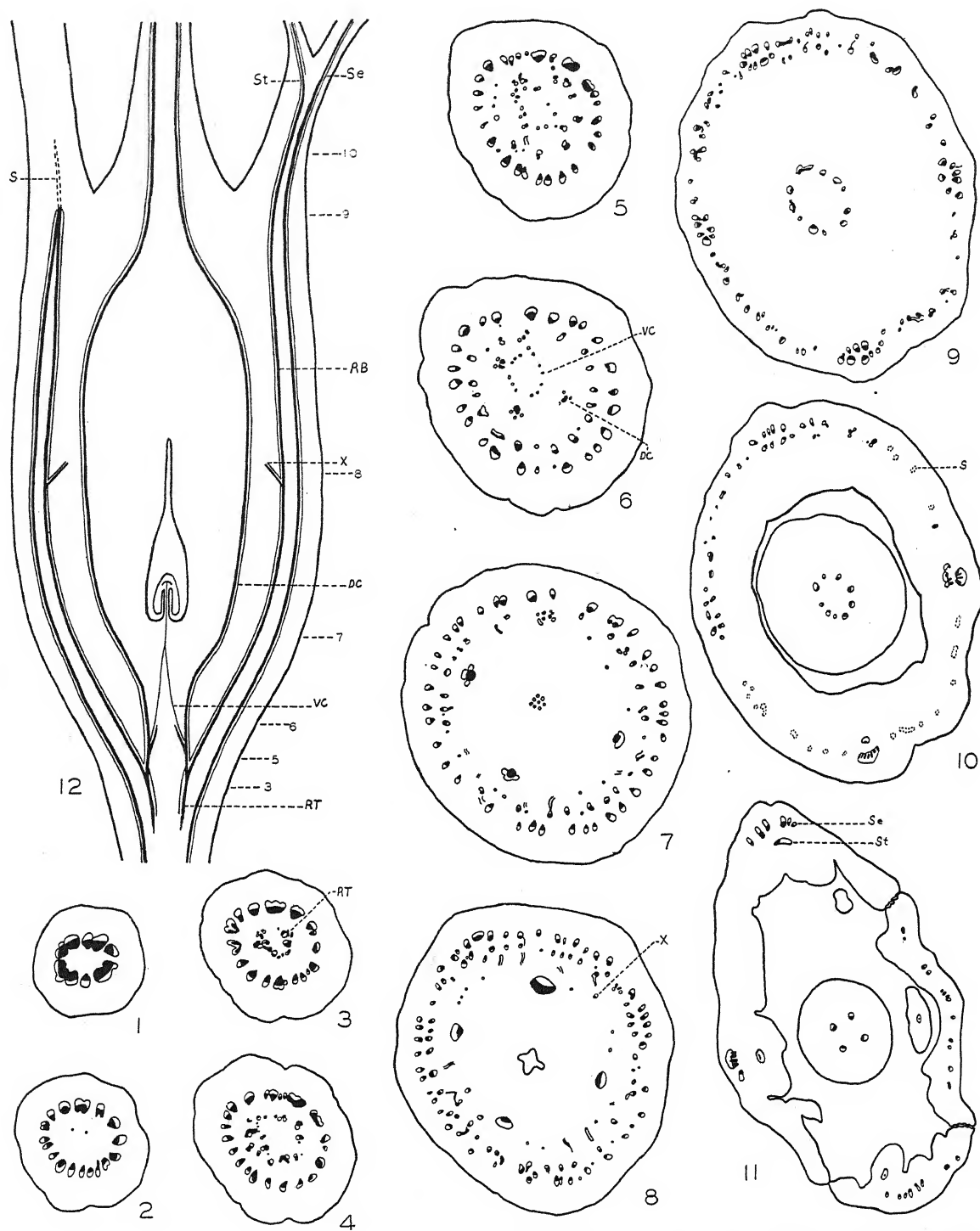


Fig. 1-12.—Fig. 1-11. Semi-diagrammatic cross sections of female flower.—Fig. 12. Reconstructed longitudinal diagram through two dorsal traces, a sepal and stamen on one side and between the sepals on the other. Sepal traces, stamen trace, dorsal and ventral carpellary traces and residual tissue are indicated by Se, St, DC, VC and RT respectively. S indicates the xylem-like sclerenchyma at base of floral tube, RB recurrent bundles. X indicates branch traces, shown cut in figure 12, which form, with dorsals, the innermost ring of bundles.

mulaceae (Engler and Prantl, 1935; Wilson and Just, 1939). In both of these families the stalk has been interpreted by various workers as consisting of either axial or floral tissues or both.

The assumption in practically all of the taxonomic literature on the Santalaceae that the inferior ovary is receptacular seems to be based principally on popular adherence to the receptacular theory, rather than on any definite anatomical evidence obtained from this family.

Santalaceae is a family of apetalous trees and shrubs occurring chiefly in the tropics. All members are generally considered to be partially parasitic on other flowering plants. *Darbya umbellulata* A. Gray (*Nestronia umbellula* Raf.) has usually four sepals with the stamens opposite the sepals and attached to them. The ovary is completely inferior with a single locule and a central placental stalk bearing 2–3 ovules at the top.

For this study flowers were obtained from herbarium sheets, boiled in water and treated overnight with 2 per cent potassium hydroxide, then dehydrated and embedded in paraffin. A very satisfactory stain was obtained by treating sections with 1 per cent chromic acid for twelve hours, rinsing in water, staining in 1 per cent aqueous safranin for twelve hours, rinsing and dehydrating, and finally treating with a dilute solution of fast green in clove oil and absolute alcohol to differentiate simultaneously the safranin and counterstain.

THE FEMALE FLOWER.—In the peduncle the vascular tissue is in the form of an almost continuous cylinder (fig. 1). At the base of the ovary this cylinder begins to separate radially into numerous strands (fig. 2). These strands swing outward and pass up the outer portion of the ovary to approximately the level where the floral tube is freed from the ovary. The number of traces in this ring of bundles is continually increased by radial separation throughout this distance (fig. 2–8). No branch traces are given off from these strands and they form few or no connecting traces among themselves. A second ring of bundles is present inside the first for the full length of the ovary. These inner traces are inverted with the xylem located outside the phloem (fig. 6–8). At approximately the level where the floral tube is freed from the ovary, the outer ring of normally oriented traces and the inner ring of inverted traces, except those in the positions of the sepals, come together in a series of anastomoses (fig. 9, and left side of fig. 12). This is the only region where there are strong connections between the two rings of bundles. At lower levels there are a few connections which are too weak and irregular to be considered as significant. Continuing upward from the connections between the two rings of bundles are a few strands of xylem-like sclerenchyma (S, fig. 10, 12) which soon disappear.

In following the bundles of the inner ring downward to the base of the ovary, it can be noted that there is a gradual reduction in number. This reduction results partly from fusion of traces and partly

from the gradual reduction in size of some traces until they disappear. The inner bundles are connected to each other at irregular intervals by small traces. Traces to the inner parts of the flower originate near the ends of the inverted traces. The relation of these branch traces to the inverted traces can best be described from the base of the flower upward. The traces of the inner ring first appear very low in the ovary and consist of a few xylem strands located in what appears to be the pith of the peduncle (fig. 2). Above this level the number of strands of the inner group increases rapidly by the appearance of new traces. Figure 3 represents a cross section taken only a short distance above the level of figure 2 and shows a ring of traces consisting of xylem only or of xylem and phloem in the inverted position. These traces soon form a complete ring which gives off branches to the inside (fig. 4, 5). There are three or occasionally four branches which are more strongly developed than the others. These are normal collateral bundles and they swing outward slightly at higher levels while the traces between them move inward (fig. 6). The traces moving inward usually consist only of phloem. Since these strands supply the ovules, they must be considered as ventral carpellary traces. If xylem is present, it is in the lower portions of the traces only and is in the normal position and not inverted as is usually the case in other families. These traces continue to move inward to form a compact strand of phloem which enters the placental stalk (fig. 7, 12). In other genera of the Santalaceae which have been studied, the placental stalk may have a large amphicribal trace, which implies a fusion of normal collateral bundles.

The larger traces, with the xylem and phloem in the typical collateral arrangement, continue to swing outward as they pass up through the ovary. At approximately the level of the top of the locule, branch traces move inward from the ring of inverted traces and take up positions between the large bundles (X, fig. 8, 12). Thus there is formed at this level a third ring of bundles, all of which in this case have the xylem and phloem normally oriented. These swing inward at the top of the ovary and form anastomoses with each other just before the style is freed (fig. 9). A varying number of traces continue upward from these anastomoses to enter the base of the style (fig. 10). The smaller ones soon disappear and only three or four traces, in the same positions as the large traces below this level, continue up into the style (fig. 11). The stigma has as many lobes as there are traces in the style, and one trace extends into the base of each lobe. Thus the large traces occupy the position of dorsal carpellary traces. This indicates that the ovary here consists of usually three but sometimes four carpels.

Directly opposite the sepals, the traces of the outer ring and the inner inverted traces do not connect at the top of the ovary as do the traces between the sepal positions. Instead they swing closer together to form what corresponds to a large compound

amphicribal bundle with the individual traces rather loosely arranged (fig. 9). However, the outer and inner traces remain distinct and at the base of the floral tube each group becomes even more compactly arranged. The inner traces opposite each sepal give rise to a single inverted trace (fig. 10). Each of these traces then swings inward to enter

trace divides radially to form the vascular bundles of the sepal (fig. 11).

THE MALE FLOWER.—The peduncle of the male flower, as of the female flower, has a compact ring of bundles which divides rapidly in radial planes to form numerous traces which enter the base of the flower. There are four lobes in the base of the cavity of the flower which probably represent four much reduced carpels (fig. 13). In some flowers a rudimentary placental stalk may also be found. A very few xylem strands may appear in the pith just below this level, but usually these are missing. Small xylem bundles may appear just inside the ring of larger traces. Some of these develop phloem on the inside to form small, inverted traces and some of the bundles in the outer ring give rise to branch traces which swing inward and rotate to assume the inverted position (fig. 13, 14). Inverted traces are more numerous and better developed opposite the positions of the sepals. Some of the small traces between the sepal positions are normally oriented and some are inverted. There is always a much smaller number of inverted traces here than in the female flower, and they are not so regularly arranged in a distinct inner ring. Just below the level where the stamen filaments begin to differentiate from the floral tube the normal and inverted traces located between the sepal-stamen complexes anastomose just as in the female flower (fig. 15). Above these anastomoses some traces continue upward for a short distance as xylem-like sclerenchyma and then disappear (fig. 16).

The stamen traces are differentiated from the inverted traces opposite each sepal (fig. 16, 17). There may be formed a single inverted trace which soon becomes amphicribal, or there may be two traces, one usually consisting of phloem only, which fuse either in the floral tube or in the base of the filament to form a single trace. In many of the flowers studied two superimposed stamens are formed opposite each sepal. Where this condition is found the stamen next to the sepal receives the larger trace and bears well developed pollen. The inner stamen trace usually consists of only a small amount of phloem, and the stamen receiving it produces aborted pollen or exists only as a staminodium.

INTERPRETATION.—The course of the principal vascular bundles in the ovary and the place of origin of the carpellary traces strongly indicate that the inferior ovary in *Darbya* is axial in nature. Both the outer ring of normal collateral bundles and the inner ring of inverted bundles must be considered as receptacular. The presence of a complete ring of inverted traces can best be explained as the result of invagination of the tip of the axis, just as has been described for *Rosa* and *Calycanthus*. This interpretation is supported by the fact that the dorsal and ventral carpellary traces arise from the inner inverted traces and not directly from the stele at the base of the ovary as would be the case if the inferior ovary were the result of extreme adnation. Also, it is to be noted that the inverted traces extend below the level at which the carpellary traces arise. The vascu-

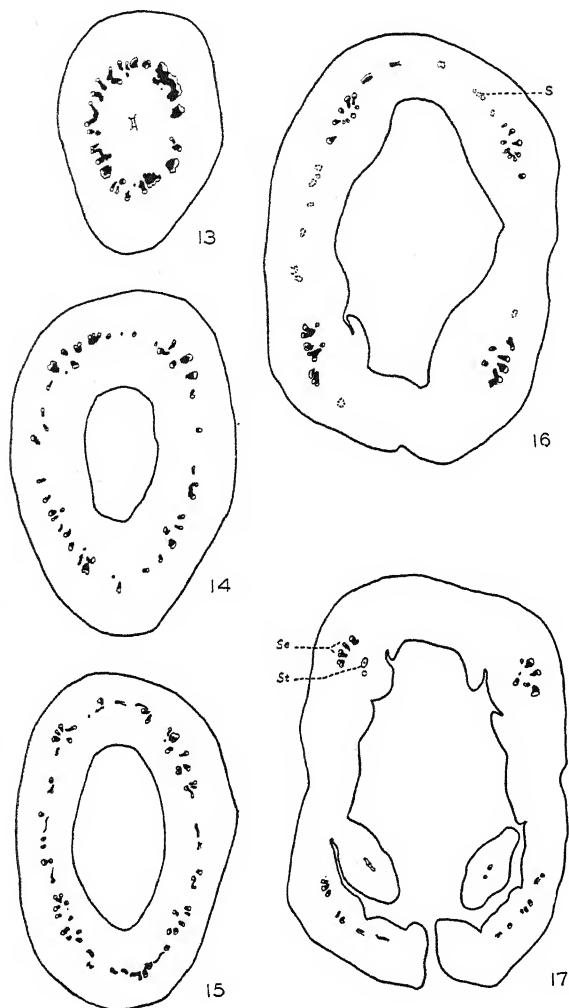


Fig. 13-17. Semi-diagrammatic cross sections of male flower. Sepal traces, stamen traces and xylem-like sclerenchyma are indicated by St, Se and S respectively.

the base of a stamen (fig. 11). Usually before it enters the stamen the trace becomes amphicribal with a very small amount of xylem. The fact that these traces become amphicribal is probably not significant, since weak traces in flowers rather frequently show this condition. The anthers of the female flowers studied contained only aborted pollen grains.

The outer traces continue as large bundles for a short distance after the divergence of the stamen traces. At approximately the level where the sepal lobes are differentiated and the stamens freed, each

lar tissue below this level is apparently comparable to the residual tissue found in many other flowers. By residual vascular tissue is meant any vascular tissue of the stele or receptacle which may remain after all traces to floral parts have been given off. Since residual tissues represent the tips of the receptacular traces, in *Darbya*, the tip of the receptacle is directed downward. As a result the traces appear to be located in the pith of the peduncle. Thus the assump-

no traces to the disc, but, if the lobes are derived from petals, then the distal portion of the floral tube consists of fused sepals, petals and stamens.

If the interpretation of the inverted traces given here is correct, then one can agree with Van Tieghem (1869) that the placental stalk must be entirely floral. If the stalk were partly axial, the residual tissue would be directed upward inside the ventral carpellary traces. No evidence was found to substan-

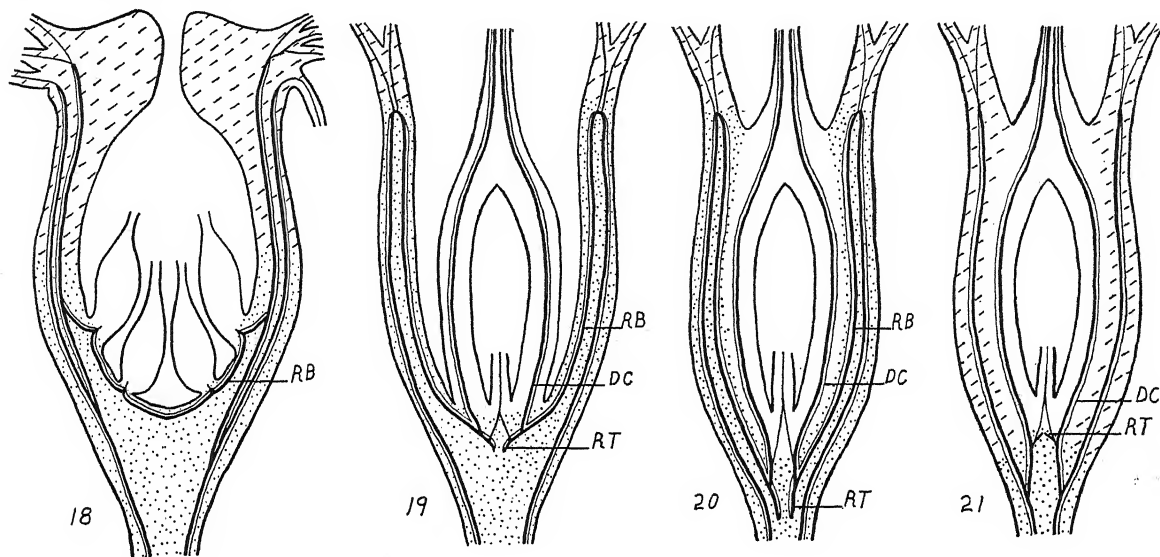


Fig. 18-21.—Fig. 18-20. Longitudinal diagrams illustrating a possible method of origin of the receptacular inferior ovary of the Santalaceae.—Fig. 18. The floral cup of *Rosa* (adapted from Jackson, 1934).—Fig. 19. A hypothetical flower with a compound pistil and a floral cup which is largely receptacular.—Fig. 20. The inferior ovary of *Darbya*, which might have resulted from the fusion to the ovary of the floral cup shown in figure 19.—Fig. 21. A hypothetical inferior ovary as it might appear had it resulted from adnation. Receptacular tissues are indicated by stippling; tissues derived from fused sepals, petals and stamens by diagonal lines; the ovary wall is unshaded and ovules are omitted. Recurrent bundles, dorsal carpellary traces and residual tissue are indicated by RB, DC and RT respectively.

tion that invagination of the receptacle has occurred is supported by the presence of the inner ring of inverted bundles and the orientation of the residual tissue.

The receptacular tissue thus surrounds the ovary and extends approximately to the level where the floral tube is freed from the ovary. This is the level where the outer traces turn downward and become inverted. Possibly the base of the floral tube also is receptacular. The xylem-like sclerenchyma strands extending into the tube from the point where the traces of the outer and inner whorls join may indicate that the recurrent portions originally extended higher in the tube. As a result of fusion at the top because of proximity, the present level where the traces turn downward may be below the original level. In that case the exact proportion of the floral tube which consists of receptacular tissue would be difficult to determine. Undoubtedly the distal region of the tube consists of fused sepals and stamens. Whether the petals are also fused into this tube cannot be stated definitely, since there are no indications of petal traces. The disc in the Santalaceae has lobes alternate with the sepals. There are

tiating this idea. That the placental stalk is composed of carpellary tissues only is also supported by the fact that of the various members of the family, there are some in which the locules are separate, some in which the locules are continuous at the top but separate at the base with a corresponding reduction of the partitions, and some in which a complete loss of the partitions results in a single locule with a placental stalk attached at its base. This interpretation of the placental stalk in *Darbya* is essentially the same as that given by Dickson (1936) and Douglas (1936) for the Primulaceae.

The interpretation of the course of the stamen traces is more difficult. In both *Rosa* and *Calycanthus* the recurrent portion of the stele is the region between the points of origin of the stamen and carpellary bundles. It seems likely that this is the case in *Darbya*, but the method and point of origin of the stamen trace is obscured by extreme variation in different flowers and even in the same flower. At the level shown in figure 9 there is a group of bundles representing a combined sepal-stamen trace. The inner bundles may become very much reduced and all but one disappear; or they may fuse to form a

single trace; or there may be weak anastomoses between them, and from the anastomoses a single stamen trace may emerge which is inverted for only a short distance and then becomes amphicribal. There may also be very weak connections with the outer bundles. This latter condition is provisionally interpreted as indicating that the stamen trace originates in the base of the floral tube. This is supported to some extent by the fact that a single trace that can be definitely identified as a stamen bundle is not evident until approximately at the level where the sclerenchyma strands disappear. As suggested previously, the extent of the xylem-like sclerenchyma strands may indicate the extent of the receptacular portion of the floral tube.

It is difficult to interpret the branch traces which extend inward from the inverted bundles and assume a position between the dorsal carpellary traces (X, fig. 8, 12). There would seem to be three possible explanations: that they represent another whorl of organs, that they are median or secondary carpel traces, or that they are merely anastomosing cross branches of no morphological significance. The third possibility seems most likely because of the anomalous position, large number and small size of the traces. On the other hand, these traces consistently appear in other genera of the family and may possibly have some significance not yet understood.

The male flower is derived from a perfect flower by reduction. The dorsal and ventral carpellary traces have disappeared completely, though they are still present in the male flowers of some other genera of the Santalaceae. There is a strong tendency toward a reduction of the inverted traces. In other genera, to be described in a later publication, these traces have disappeared completely. Aside from these reductions the male flower may be interpreted as having the same structure as the female flower.

DISCUSSION.—The evidence presented here indicates that the inferior ovary in *Darbya* originated by invagination of the floral axis and subsequent fusion of the resultant cup-shaped receptacle to the ovary. Thus the outer tissues surrounding the ovary, and probably the base of the floral tube, are axial or receptacular. A complete progressive series illustrating this fusion is not available, but the situation described for *Rosa* and other genera of the Rosaceae by Bonne (1928) and Jackson (1934) and for *Calycanthus* by Smith (1928) could well represent the beginning of such a series. Figure 18 shows the structure of the floral cup of *Rosa*, with the receptacular portion indicated by stippling and the appendicular portion by diagonal lines. Progenitors of the Santalaceae may have had a floral structure similar to this. There probably followed a fusion of simple pistils in the bottom of the floral cup to form a compound pistil, and an increased invagination resulting in the hypothetical flower illustrated in figure 19. The subsequent fusion of the axial portion of the cup to the ovary would result in the situation described for *Darbya* (fig. 20). Thus the tissues surrounding the ovary would be receptacular, with re-

current traces, while the tube extending beyond the ovary would consist of axial tissue in the proximal regions and of fused floral parts in the distal regions. It must not be implied from this discussion that either the Rosaceae or the Calycanthaceae are considered to have necessarily given rise to the Santalaceae. There are several families in which cup-shaped floral tubes are found that have not yet been investigated by the floral anatomist.

The residual tissue in figures 19 and 20 is directed downward into the pith of the peduncle. Figure 21 illustrates the structure that might be found in an inferior ovary derived by adnation of floral parts. There is no indication of recurrent traces and the residual tissue is directed upward. Thus while the ovaries illustrated in figures 20 and 21 are superficially the same, the course of the vascular bundles indicates that the former is receptacular and the latter is appendicular.

While it has been held for some time that an inferior ovary may be surrounded by tissues of the receptacle, the theory has not been supported by evidence acceptable to most plant anatomists. Most of the genera used in evidence have been shown rather conclusively to have inferior ovaries which resulted from extreme adnation, and hence are appendicular in nature. For example, members of the Rosaceae have been widely used by supporters of the axial theory, but the evidence supporting the appendicular theory is most complete and convincing in this family. This evidence seems too complete not to be accepted.

If the interpretation given here for *Darbya* is correct, it then follows that both axial and appendicular inferior ovaries occur, and not only one or the other as has been rather generally assumed. There is, of course, the distinct possibility that a third type which is axial below and appendicular above may also occur. A completely inferior ovary of this type has not yet been described. It is entirely probable that receptacular inferior ovaries have developed in other dicot families just as the floral cup which is axial below and appendicular above has appeared independently in the Rosaceae and Calycanthaceae. The inferior ovary in most dicot families is undoubtedly derived by extreme adnation as has been shown particularly by Eames. There seems to be no possibility that the type of inferior ovary described here could have given rise to the type which has been interpreted as having arisen by adnation, and certainly the reverse is true. Thus the origin of the inferior ovary in each family must be determined by critical anatomical study and not by superficial comparison with some apparently related form.

Many variations in the structure of inferior ovaries of a floral nature have been described by other investigators. Obviously variations in receptacular inferior ovaries are to be expected from evolutionary changes. From a study of over sixty-five species representing more than thirty-five genera of the Santalaceae and related families, it seems likely that in *Darbya* there is a somewhat primitive condition.

There is great variation in the floral anatomy of this group and most, if not all, variations in structure can be explained as derivatives from the *Darbya* type. Without a knowledge of the relatively primitive structure of *Darbya*, it is difficult to interpret the floral anatomy of the forms in the Santalaceae studied by previous investigators, notably *Comandra*, *Thesium* and *Osyris*. A discussion of the variations in other genera will appear in a later paper (Smith and Smith, in manuscript).

SUMMARY

The vascular anatomy of both male and female flowers of *Darbya* is described.

The vascular bundles of the receptacle in the female flower extend from the peduncle to the top of the ovary before any traces to floral appendages appear. At this level some of the traces continue into the sepals. The remainder of these traces then turn downward to form an inner ring of recurrent, inverted bundles reaching to or below the base of the ovary. The dorsal and ventral carpellary traces are derived from these inverted strands at the base of the ovary. A small amount of residual vascular tissue extends downward beyond the point of origin of the carpellary traces. The inverted traces opposite the sepals give rise to the stamen traces.

The presence of recurrent inverted bundles, the downward extension of the residual tissue and the

origin of the carpellary traces from the inverted strands are interpreted as indicating that the inferior ovary here originated by invagination of the tip of the floral axis. Hence the outer tissues surrounding the ovary, containing the ascending and recurrent traces, are axial or receptacular. The basal portion of the tube extending beyond the ovary is probably composed of axial tissues while the upper portion represents fused floral parts.

Since the residual tissue is directed downward, the placental stalk is interpreted as consisting entirely of carpellary tissue.

The male flower may be interpreted in the same manner as the female flower except that the carpellary traces and many of the inverted traces have been lost by reduction.

It is suggested that anatomical evidence obtained thus far indicates that there are at least two types of inferior ovaries. One type, the result of extreme adnation, is entirely floral in nature. The other type, described here, would seem to have resulted from invagination of the floral axis followed by fusion to the ovary of the receptacular portion of the resultant cup.

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CULTIVATION IN VITRO OF SMALL DATURA EMBRYOS¹

J. van Overbeek, Marie E. Conklin and A. F. Blakeslee

WHILE ATTEMPTING to induce artificial parthenogenesis by injecting physiologically active substances into unfertilized ovaries of *Datura stramonium* (16) it was realized that some of these substances might never penetrate the ovules far enough to reach the egg cell. With this in mind experiments were planned by which the development of the egg cell and the subsequent parthenogenetic embryo, should such be obtained, could take place *in vitro*. In order to accomplish this, two major steps are necessary: (a) division of the egg cell must be induced, and (b) development of the resulting embryo into a viable plant must be accomplished. It seemed obvious to try to control step (b) before attempting (a). The present paper deals with step (b), the culture of small embryos *in vitro*.

Successful culture of embryos has been reported in the literature (10, 14, 20). For a review of the literature, see LaRue (10) and White (19). The media used by the different authors vary considerably. Tukey (14) concluded that there was no appreciable difference in effect between the different salt solutions used by various workers. The concentration, too, could be varied from one to ten times without much effect. It seems clear, however, that the necessity of adding organic substances to the medium greatly depends upon the stage at which the embryos are isolated. Thus mature embryos can be grown in a strictly inorganic medium. The addition of sugar even retards their growth (14). Nearly mature embryos require sugar (10, 14). According to Tukey, the younger stages require more than the older ones. Still younger embryos require, in addition to sugar, the heat stable factors found in yeast extract or fibrin digest (10, 20). LaRue employed indoleacetic acid in low concentrations to replace yeast extract in his media.

Although the embryos successfully cultured by the authors mentioned above were often very immature, they still were isolated at a relatively advanced stage of development. LaRue (10), who successfully grew the embryos of a large number of species, makes the following statement: ". . . embryos 0.5 mm. or slightly less in length can be grown successfully . . . those of still smaller size have not yet been brought under control. . . . at a length of 0.5 mm. we may have reached a new lower threshold which will be difficult to pass." It is well to remember that the actual size of an embryo is not a measure of the stage of development in different species. Thus an embryo 0.5 mm. long of a plant having relatively large embryos, such as *Datura*, will be in a much earlier stage of development than an embryo of the same length of a plant such as *Nicotiana*, which has minute embryos.

White (20) isolated young heart-shaped embryos from *Portulaca*. These embryos grew from 0.12 to

1.84 mm. in three weeks and were not shown to be viable. A personal communication from investigators at the University of Wisconsin states that they are currently engaged in the problem of isolating and growing young embryos *in vitro*. Howard E. Heggestad (Univ. of Wisconsin, unpublished data) has succeeded in isolating embryos of *Nicotiana rustica* only nine days after pollination. Using vitamins B₁, B₂, B₆, nicotinic acid and pantothenic acid in addition to the medium used by White, he obtained viable seedlings. Lewis T. Ausherman (Univ. of Wisconsin, unpublished data) has successfully cultivated a presumably hybrid embryo of *Hordeum jubatum* × *Secale cereale*, which was taken out eleven days after pollination. The medium contained yeast extract.

Since it appeared that young embryos are less autotrophic in respect to growth factors than older ones, and since in the ovule immature embryos are nourished by the endosperm, it occurred to us that some form of endosperm should be added to the culture medium in order to supply the necessary growth factors. Coconut milk was used for this purpose. This addition made it possible to cultivate *in vitro* embryos of *Datura stramonium* so immature that they were of small heart shape and only 0.15 mm. in length (the embryo in the mature seed is approximately 6 mm. long). In many cases such embryos increased in length from 0.15 mm. to 6.0 or even 8.0 mm. after seven days of cultivation *in vitro*. They subsequently developed, by proper treatment, into normal plants.

NORMAL DEVELOPMENT OF DATURA EMBRYOS.—In order to understand the development of embryos *in vitro*, a brief outline of the normal development of *Datura* embryos follows. Detailed work by Satina and Blakeslee on the histology of the normal development of *Datura stramonium* is in progress. About thirty hours after pollination, fusion of the gametes occurs. Nine to fourteen days following pollination, the embryo has developed into an undifferentiated knob-like structure attached with the suspensor at the micropylar end of the embryo sac. According to the terminology of Souèges, the embryo at this stage is called a *proembryo* because it shows axial symmetry. At a later stage the apical part flattens out, due to formation of the primordia of the cotyledons. When they start to grow out, a *heart-shaped* embryo results. Such heart-shaped embryos may be found between ten and sixteen days after pollination. Embryos in this particular stage of development, varying between 0.15 and 0.5 mm. in length, were extensively used in these investigations. When the embryo reaches a length of 1 mm. or over it assumes a *torpedo shape*. At this stage the cotyledons are about half as long as the total embryo. A root cap and also the meristem of the epicotyl has been initiated. When the embryo is about 2 mm. long, it begins to curve. At still later stages it becomes folded inside the seed, and at maturity is about 6 mm. long.

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We wish to express our appreciation to Mr. George Risman for his skillful assistance.

TECHNIQUE OF ISOLATING EMBRYOS.—The embryos were grown under aseptic conditions. The capsules of field-grown plants were dipped in alcohol, soaked in 0.1 per cent HgCl_2 for a few minutes, rinsed with sterile water and cut in a sterile Petri dish. The ovules were removed and held between two glass slides which were sterilized immediately before using by dipping in alcohol and flaming. Next the ovules were cut longitudinally by drawing a flamed razor blade between the slides. It was found that by using the blade while it was still warm cutting was facilitated. When proembryos or heart-shaped embryos

less satisfactory than low percentage agar media. This is in agreement with Tukey (14) and LaRue (10). Tukey, however, claimed better results with embryos placed on the surface. This disagreement with our observations might be explained on the basis of a difference in stage of development of the embryo at the time of isolation. Tukey's embryos were more mature. White (22) reported differentiation in growth of callus tissue placed below the surface of a liquid medium.

MEDIA AND RESULTS.—Embryos in stages ranging from medium curved (fig. 1a) to mature embryos

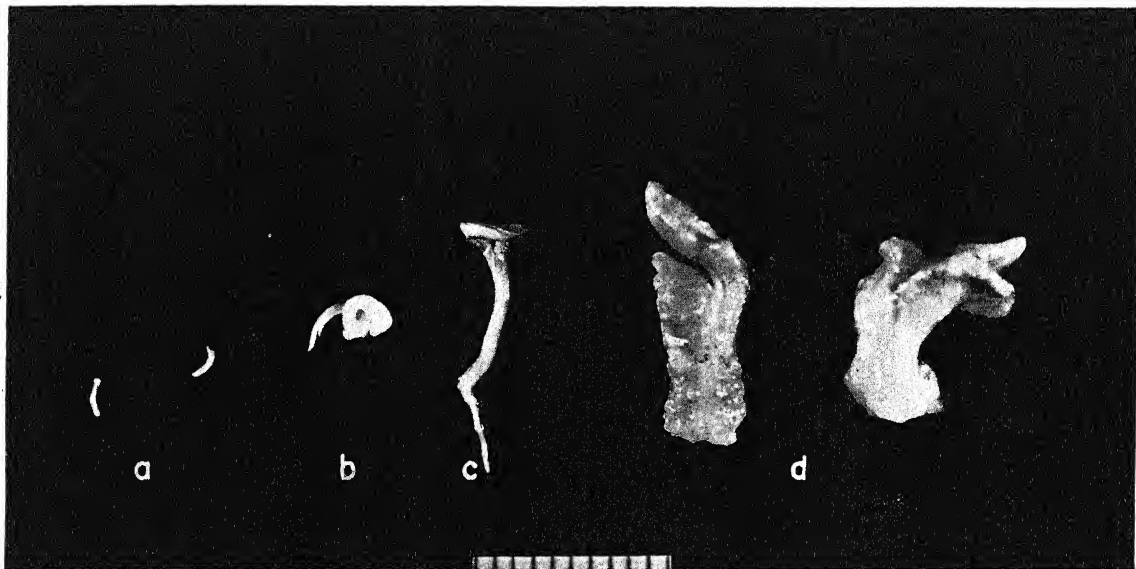


Fig. 1. Effect of different media on growth *in vitro* of *2n* *Datura stramonium* embryos. Initial size 2 mm. fourteen days after pollination. Photographed after eight days in incubator at 25°C. and an additional nine days in the greenhouse. Embryos shown came from the same capsule. (a) On plain agar—no growth; (b) agar with mineral salts and sugar only—no cotyledons, not viable; (c) on medium as (b) but with mixture of growth factors added (see text)—complete, viable seedling; (d) on medium similar to (c) but with non-autoclaved coconut milk added—no roots, enlarged cotyledons and hypocotyl. Scale in mm.

were to be isolated, the ovules were cut into almost equal parts, but when larger embryos were to be removed they were cut into unequal parts so as to avoid damage to the embryo. If the ovule was correctly cut, the embryo could be seen in one of the cut parts of the ovule when inspected under the preparation microscope. The embryos could be removed without injury under the preparation microscope by means of sterile needles. Fluorescent lights (two 40-watt bulbs) were used for illumination because the heat of ordinary incandescent lights caused the embryos to dry out too rapidly. The embryos were transferred to sterile vials (35×10 mm.) containing $\frac{3}{4}$ cc. of an agar medium described below. The embryos were placed below the surface of the medium, because they grew more uniformly this way than when placed at the surface. The cultures were kept in an incubator at $25^{\circ} \pm 1^{\circ}\text{C}$. Some cultures were transferred to the greenhouse after they had been in the incubator for approximately one week. Liquid media were found

usually could be grown in a medium consisting of 1 per cent agar (in later experiments 0.8 per cent was used), 1 per cent dextrose and mineral salts mixed according to Tukey (14, 15). When kept in dim light, a root, the hypocotyl and small cotyledons usually developed and viable seedlings were obtained. Embryos at "torpedo" stage developed a root and a hypocotyl but usually failed to develop cotyledons on this medium (fig. 1b). In order to induce better growth, such young embryos were grown on a medium to which in addition to the ingredients already mentioned, a mixture of growth factors was added.² This mixture was arbitrarily made up and, because it proved effective, was maintained throughout this investigation. It is highly probable that some of the compounds are not essential and that the essential ones are not present at optimal concentra-

² Concentrations in mg. per liter; glycine 3.0, thiamin 0.15, ascorbic acid 20.0, nicotinic acid 1.0, vitamin B₆ 0.2, adenine 0.2, succinic acid 25.0, pantothenic acid 0.5.

tions. Glycine was chosen because according to White (21, 23) it promotes growth of tissue cultures. There is, however, considerable disagreement as to its essentiality for root growth (5, 12, 8). Thiamin is a well-known growth factor for embryos (9, 4) and roots growing *in vitro* (3, 11). Ascorbic acid is also a growth factor for plants (6). Nicotinic acid and vitamin B₆ are recognized as growth factors for some roots (1) including those of *Datura* (5). Adenine is a leaf growth factor (2). Succinic acid, ac-

cording to Thimann (13), is essential for auxin-controlled respiration. In this medium embryos, when isolated at torpedo stage and kept in dim light can be made to develop into viable seedlings (fig. 1c) with well-developed cotyledons. However, when the embryos were below 0.5 mm. in length when isolated, they failed to develop in this medium (fig. 6c). This agrees with the limit set by LaRue (see above).

In order to promote the growth of embryos below 0.5 mm. in length, a new medium was devised. Since



Fig. 2-5.—Fig. 2 (upper left). Growth of $2n$ embryos, initially heart-shaped, 0.2 mm. largest dimension, isolated ten days after pollination. Photographed after eight days in incubator at 25°C. and an additional five days in the greenhouse. (a) On agar medium containing sugar, mineral salts and vitamin mixture; (b, c and d) three tests made with a medium similar to (a) to which non-autoclaved coconut milk from three different coconuts was added. Scale in mm. Note branched and lobed cotyledons in some cases.—Fig. 3 (upper right). Test similar to figure 2. The heart-shaped embryos initially 0.25 mm., isolated ten days after pollination. Photographed after eight days at 25°C. (a) On medium similar to figure 2a—no growth; (b, c, d) three tests made with a medium similar to (a) to which non-autoclaved coconut milk from three different coconuts was added—growth and differentiation of hypocotyl and cotyledons.—Fig. 4 (lower left). Growth of $4n$ embryos, initially 0.3 mm. greatest dimension, heart-shaped and isolated eleven days after pollination. Photographed after eleven days at 25°C. (a) On agar medium containing mineral salts, sugar and vitamin mixture—no growth; (b) on same medium to which autoclaved coconut milk was added—callus-type growth; (c) on medium same as 2a to which non-autoclaved coconut milk was added—growth and differentiation of hypocotyl and cotyledons.—Fig. 5 (lower right). Growth of $2n$ embryos initially pro-embryo to very small heart-shaped 0.14 in diameter, isolated fourteen days after pollination: (a) indicates original size, (b) photographed after twelve days at 25°C. on medium similar to figure 4c—growth and differentiation of hypocotyl and cotyledons. Note plumule development.

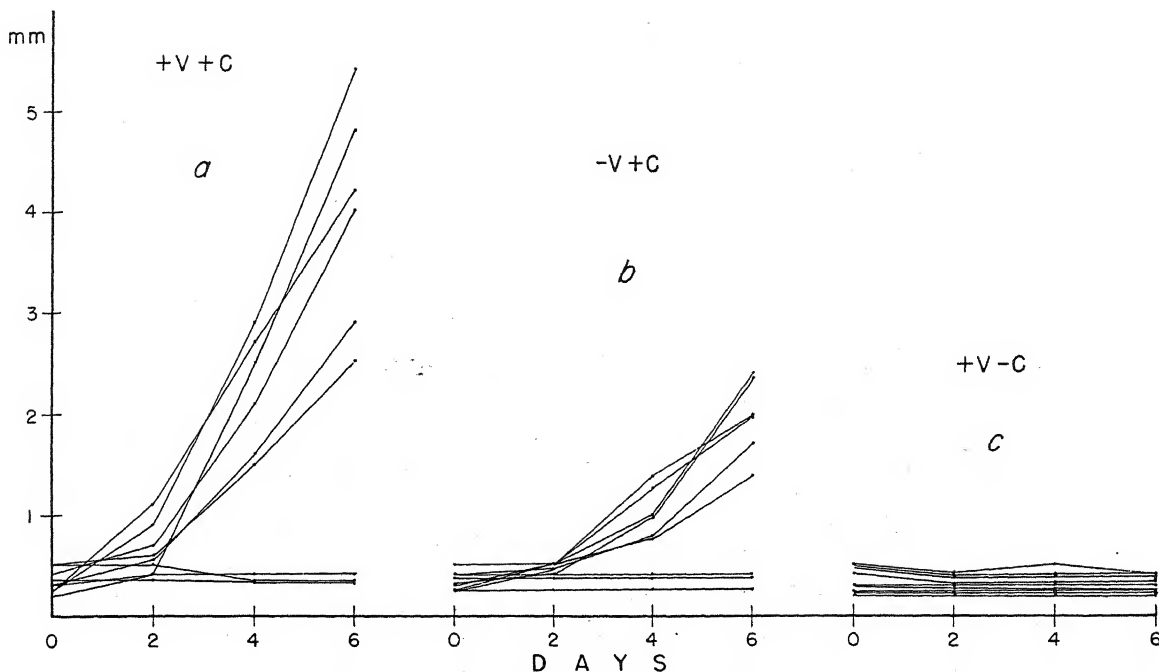


Fig. 6. Growth curves of individual embryos isolated when heart-shaped, twelve days after pollination. Ordinate: length in mm. Abscissa: days of cultivation at 25°C. (a) Agar medium containing sugar, mineral salts, vitamin mixture (see text) and non-autoclaved coconut milk; (b) similar to (a) with vitamin mixture omitted; (c) similar to (a) with coconut milk omitted.

in nature embryos are nourished by the endosperm, a natural endosperm in the form of coconut milk was added to the medium already containing agar, dextrose, inorganic salts and the mixture of physiologically active substances mentioned above. The coconut milk was removed under sterile conditions by pipetting and stored in Erlenmeyer flasks. The activity of the coconut milk stored in this manner in the refrigerator was maintained in most cases for at least several weeks. When one part of sterile non-autoclaved coconut milk was mixed with approximately two parts of the still-warm agar medium, heart-shaped embryos as small as .15 mm.³ in largest dimension could be grown (see table 1). The rate of growth amounted to several hundred times increase in volume per week (17). In one case even radially symmetrical embryos were grown (fig. 5), although later attempts to repeat this failed. Autoclaved coconut milk was not effective, but occasionally supported a callus-like type of growth without much differentiation (fig. 4b).

Figure 6 shows the growth in length of heart-shaped embryos which were between 0.2 and 0.5 mm. long when removed from the ovules. When grown in the complete medium (fig. 6a) containing both the mixture of growth factors and non-autoclaved coconut milk,⁴ noticeable growth occurred within two

³ Since the measurements of the embryos immersed in agar were made through the curved glass wall of the culture vial, the actual length is slightly less.

⁴ The coconut milk used in this particular experiment had been stored in the refrigerator for ten days.

TABLE 1. Length (in mm.) of small heart-shaped *Datura* embryos, which were 0.15 mm. in length when isolated, grown on a medium containing 0.2 cc. of non-autoclaved coconut milk per 0.75 cc. of agar medium, at 25°C. Data selected from material of two large experiments. Controls grown on medium without coconut milk shrank slightly.

	3 days	4 days	7 days
..		1.5 ^a	5.0 ^a
..		1.05	1.6
..		2.0	4.2
..		2.5	4.6
1.45 ^a	..		2.5
0.8	..		2.6
0.9	..		6.0
1.2	..		4.5
1.3	..		8.0
1.6	..		6.0
1.65	..		6.0

^a See footnote 3, p. 475.

days, and after six days some of the embryos were 5 mm. long and 0.8 mm. in diameter. It should be noted that in figure 6 the growth curves of individual embryos are represented. All embryos were removed from ovules contained in one and the same capsule. It is clear that even in the complete medium not all embryos developed. Three of the nine embryos of figure 6a failed to grow. This failure is not associated with a possible small initial size of the embryo, be-

cause the three smallest embryos, which were respectively 0.2, 0.25 and 0.25 mm. long, gave the largest embryos after six days of cultivation (respectively, 4.8, 4.2 and 5.4 mm. in length and 0.8, 0.6 and 0.8 mm. in diameter). When the mixture of growth factors was omitted from the medium, but the coconut milk maintained (fig. 6b), the embryos grew, but not as much as in the complete medium. When only coconut milk was omitted from the complete medium (fig. 6c), no growth resulted with these small-sized embryos. Similar results are shown in figures 2, 3, 4 and 5.

DISCUSSION.—A feature of the embryos grown in media containing coconut milk is the absence of roots. This is probably due to a root inhibitor in the coconut milk. This inhibitor appears to be heat stable, because, when older embryos were grown on media containing autoclaved coconut milk, root development was markedly suppressed. It is possible that the root inhibitor is related to auxin, since, despite the root suppression, growth of the hypocotyl is not inhibited by it. It is known that auxin inhibits root growth in concentrations which still permit stem growth (18). Root growth could be induced by transferring embryos which had been grown for approximately one week on a medium containing coconut milk, to a medium free of it. When placed in dim light, viable seedlings resulted which grew in soil. Embryos started on a medium such as used in the test represented in figure 6b often produced better roots than those started in the complete medium. In the course of investigations on the chemistry of the growth factors of the coconut milk,⁵ several other methods have been discovered which produced roots on the embryos without transferring. They will be reported on later.

It is interesting to note that in the normal development of coconuts the roots of the germinating coconut embryo remain in the fibrous outer shell but do not penetrate into the milk as one might expect on the basis of the position of the embryo in the seed (7). It seems likely that the root inhibitor in the milk is responsible for the failure of the roots to grow into the milk.

Another feature of the medium containing non-autoclaved coconut milk is the development of large, thick cotyledons (fig. 1d), which often show lobe formation and branching (fig. 2b, c, d). In this connection it is noteworthy that in germinating coconut embryos the cotyledons penetrate into the milk and develop into a large bulbous tissue. Again the same substances present in the milk that stimulate cotyledons of the *Datura* embryos to such a great development (fig. 1d) may also be responsible for the development of the coconut cotyledons. Often in the *Datura* experiments the plumule developed prematurely (fig. 5b).

In an attempt to simplify the technique by placing halved ovules containing the young embryo in the nutrient medium, it was found that such embryos did

not grow, whereas similar embryos, when completely isolated, grew well in the medium as shown above. A considerable callus formation usually took place at the cut surface of such halved ovules.

From the data presented one could tentatively conclude that at least three factors or groups of factors present in coconut milk affect the growth of embryos *in vitro*: (1) a thermolabile factor which causes the embryos to grow rapidly and to differentiate. In its impure state, this factor is heat stable to only about 50°C.;⁵ (2) a heat stable factor which causes a callus-like growth but no differentiation; and (3) a factor which appears to be heat stable which inhibits root development. From the fact that coconut endosperm affects the growth of *Datura* embryos one could conclude that the growth factors involved may be widespread in occurrence and of fundamental significance in the growth process of plants.

SUMMARY

Isolated heart-shaped *Datura stramonium* embryos as small as 0.15 to 0.2 mm. and as young as ten days after pollination were grown *in vitro* under aseptic conditions in an agar medium containing dextrose, mineral salts, a mixture of physiologically active substances and non-autoclaved coconut milk. At 25°C. the length of the embryos may increase from 0.2 mm. to 5 mm. within six days, corresponding to a 500 times increase in volume (fig. 6a). In one case radially symmetrical embryos 0.14 mm. in diameter grew to 10 mm. in length in ten days and increased in volume 8,000 times (fig. 5). Viable seedlings could be obtained by transferring after one week to a medium without coconut milk, which allowed the roots to develop.

It appears that in coconut milk at least three factors or complexes are present which affect the growth of embryos: (1) a thermolabile factor causing both growth and differentiation (fig. 2, 3, 5); (2) a heat stable factor causing in some cases a callus-like growth but no differentiation (fig. 4b); and (3) a heat stable factor which inhibits root growth and which may be related to auxin.

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DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

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Note that thin black lines hold up fairly well in reduction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. Keep the shading rather open. The degree of reduction needs to be known before the drawing is inked in.

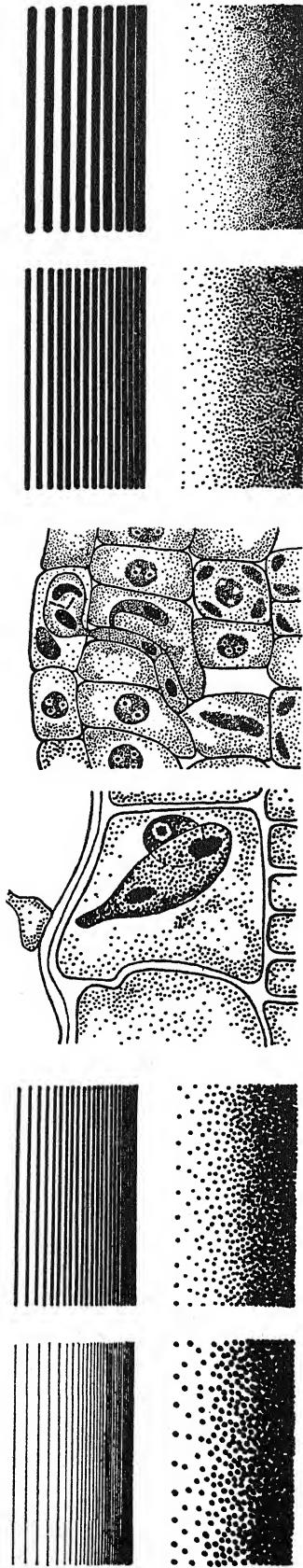
Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

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CYTOPLASMIC INCLUSIONS IN THE GLANDULAR EPITHELIUM OF THE SCUTELLUM OF *TRITICUM SATIVUM* AND *SECALE CEREALE*¹

John A. O'Brien, Jr.

ALTHOUGH THERE have been many attempts in recent years to determine the structural basis of secretion in animal gland cells, there has been very little investigation of the gland cells of plants in which modern cytological techniques were employed. While there is some agreement among biologists as to the rôles of the Golgi apparatus and mitochondria in relation to the secretory activity of animal cells, the very existence of the Golgi material in plants is questioned, and there is as yet no agreement as to the possible homologue of the apparatus in the vegetable cell. The mitochondria in plants have also been assigned a number of different functions, so it becomes highly desirable that plant gland cells be investigated further. Previous research on these cells has been confined principally to the proteolytic-enzyme-secreting cells of the carnivorous plants and to the epithelial gland cells of the scutellum of the grains. The present work is limited to an investigation of the morphological changes occurring within the cells of the latter, particularly with respect to the mitochondria. It is to be recalled that the cells in question have a two-fold function, absorption and secretion, a distinction that the majority of previous workers failed to make.

Plant physiologists early advanced evidence that the scutellar epithelial cells were gland cells, although the endosperm from which the embryo had been removed could itself digest its stored starch. The glandular nature of these cells has been generally accepted and is supported by more recent investigations of the physiology of germinating grains (Grünfeld, 1926; Schander, 1934).

Cytoplasmic granules have been reported in the epithelial cells of the scutellum of barley by Brown and Morris (1890) and of corn by Torrey (1902) and Reed (1904). These investigators suggested, directly or indirectly, a similarity between the granules and the zymogen granules of animal gland cells. Guilliermond (1908) observed an increase in basophilic granules in the epithelial cells of rye, barley, maize and wheat during germination and suggested a possible correlation between their presence and secretory activity. He later (1933) maintained, however, that the secretion of diastase produced no appreciable cytological alterations in the epithelial cells, and that the basophilic granules were merely mitochondria altered by fixation. Horning and Petrie

(1927) observed an increase in the number of mitochondria by division in the epithelial cells of maize, wheat and barley, both in fixed cells and in living cells stained with Janus green. On the theory that mitochondria are the site of enzyme synthesis, they made the interesting suggestion that these bodies with the associated enzymes actually migrate from the epithelial cells into the endosperm, where, in contact with the starch grains, they disappear as the enzymes are liberated. As the identification of mitochondria in living plant cells by vital staining is very uncertain and as Janus green, together with most basic dyes, stains certain types of plant vacuoles (Bailey and Zirkle, 1931), the nature of these migratory inclusions seems somewhat uncertain. Newcomer (1938) demonstrated the presence of polymorphic and filamentous mitochondria in the epithelial cells of germinating maize but could find no evidence of their migration from the cell.

MATERIALS AND METHODS.—The plants investigated were *Triticum sativum* and *Secale cereale*. The grains were germinated in the dark on moist filter paper in Petri dishes. Since no attempt was made to keep the germinating grains at a constant temperature, the several stages of germination were identified by relative lengths of the radicle and the shoot rather than by the length of time which had elapsed since the germination was initiated. Observations were confined to fixed sections.

Zirkle (1929) suggested the use of the term mitochondria to include all those small cytoplasmic inclusions preserved by bichromates on the basic side of pH 4.2–5.2 and destroyed by more acid fixatives and by mixtures of bichromates and acetates. The pH range has since been narrowed to 4.8–5.2. This definition, used frequently in the literature, includes plastid primordia. In the epithelial cells under investigation, however, those inclusions which were observed to give rise to plastids are sufficiently differentiated in size and shape from the non-plastid forming mitochondria to warrant their designation as plastid primordia. In the present investigation, therefore, the term mitochondria is limited to those bodies included in the above definition which were not observed to give rise to plastids. The term plastid is applied only to those inclusions containing starch. The bichromate fixative used was Zirkle's (1934) modification of Erliki's potassium bichromate-copper sulfate mixture, composed of 1.25 gm. $K_2Cr_2O_7$; 1.25 gm. $(NH_4)_2Cr_2O_7$; 1.00 gm. $CuSO_4$ and 200.00 gm. H_2O . To this mixture enough pyridine was added to make a 0.50 per cent solution. The resulting precipitate was allowed to settle before the fixative was used.

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The author wishes to express his sincere gratitude to Professor Conway Zirkle for his suggestions and helpful criticisms during the course of this investigation and to Professors J. R. Schramm and William Seifriz for criticism of the manuscript. Part of this investigation was made during the tenure of a Harrison Fellowship in botany at the University of Pennsylvania.

For observations on the vacuole and its contents as well as the mitochondria, the above mixture, to which was added, in place of pyridine, 40 per cent formaldehyde to make 4 per cent, was employed. For the same purpose Bensley's (1910) formalin-bichromate-sublimite mixture was used in the following proportions: 2.50 gm. $K_2Cr_2O_7$; 5.00 gm. $HgCl_2$; 90.00 gm. H_2O . To this solution was added 40 per cent formaldehyde to make 4 per cent. After fixation in these last two solutions, the mitochondria did not retain the stain in the degree that they did after treatment in modified Erliki's-pyridine mixture, and in many sections they could not be observed. For

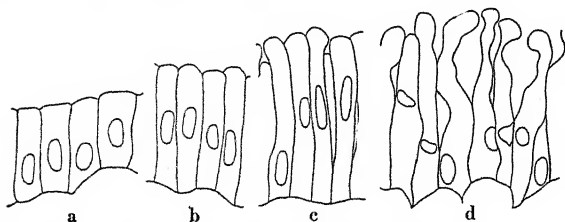


Fig. 1. Longitudinal sections of epithelial cells in grains of *Triticum* at successive stages of germination showing changes in cell size and shape; a, cells in resting grain; b, cells in germinating grain, the shoot of which has lengthened to 30 mm.; c, cells in grain with shoot 75 mm. in length; d, cells at end of germination; shoot 150 mm. in length. Irregular shape and lateral separation are evident. All figures $\times 300$.

preserving the vacuole and its contents alone, a chromic sulfate-formalin mixture was used in the following proportions: 5 gm. $Cr_2(SO_4)_3 \cdot 15 H_2O$; 0.5 gm. CuO ; 90.00 gm. H_2O . To this solution was added 40 per cent formaldehyde to make 4 per cent (Zirkle, 1928).

Hardened endosperm made sectioning difficult. The young plants with scutella intact were, therefore, removed from the grain before fixation. The specimens were placed in the fixatives for forty-eight hours, after which they were washed in water and dehydrated in butyl alcohol (Zirkle, 1930) and embedded in paraffin. Sections were usually cut 5 micra in thickness. Heidenhain's iron-alum hematoxylin was the only stain employed.

Illustrations were made with the aid of a camera lucida.

CYTOPLASM OF THE EPITHELIAL CELLS AND ITS INCLUSIONS.—As germination progresses, there is a gradual increase in the length of the epithelial cells of the scutellum accompanied by a separation of the lateral walls as was first described by Brown and Morris (1890) and substantiated by later workers (fig. 1). Greater surface exposure is thereby provided for secretion and absorption, the specialized functions of these cells. In both rye and wheat, a greater lengthening occurs in the cells in the anterior or shoot region of the scutellum. Since the embryo is situated in the posterior portion of the grain, that part of the epithelial layer opposite the radicle is regarded as the posterior region and that part opposite the plumule, the anterior region. In the later

stages, when the endosperm is approaching depletion, the epithelial cells appear as elongated, irregular projections, many of which are almost entirely separated from all adjoining cells (fig. 1d). The distal end assumes a variable form, sometimes developing what Horning and Petrie (1927) described as hypha-like protuberances (fig. 1d). It is to be noted that the lengthening of cells could be correlated only broadly with any particular stage in the germination process. Thus, occasionally, the epithelial cells of one grain are longer and display more advanced morphological changes than the epithelial cells of another grain with a shoot of equal length.

RESTING STAGE.—Aleurone grains.—In the resting stage of rye and wheat grains, the short, closely placed epithelial cells contain a very dense and usually non-vacuolate cytoplasm. All the fixatives used disclose the presence of numerous, small, clearly delineated bodies dispersed in the cytoplasm (fig. 2). These structures stain lightly and irregularly and probably represent a fixation image of the aleurone grains which Guilliermond (1908) demonstrated to be present in the cells. The fixatives used also disclose in the scutellar parenchyma of wheat and rye larger aleurone grains similar to the grains in the epithelial layer. Occasionally a few epithelial cells in a section contain grains stained intensely. More generally, however, the stain is retained only slightly so that in many cases, particularly after fixation in Bensley's mixture or chromic sulfate-formalin fixative, an internal differentiation is observed in which a dark periphery inclosing non-stained areas can be discerned. Guilliermond identified the darkly staining portion as the amorphous protein of the aleurone grain while the non-staining areas represent globoids inclosed within the grain.

Mitochondria.—The presence of mitochondria in the epithelial cells cannot be demonstrated with any certainty during the resting stage. The dense cytoplasm and the dispersion of numerous small aleurone grains make delineation of any such bodies difficult. Horning and Petrie (1927) and Newcomer (1938) reported the presence of granular mitochondria in the resting stage of maize, the former after using an osmo-chromic fixative, the latter after fixing in modified Erliki's solution. That most of the inclusions represented in figure 2 are not mitochondria can be demonstrated readily by fixing the cells in a solution which will not preserve mitochondria. For this purpose a mixture of 0.5 per cent chromic acid and 1 per cent acetic acid was used. In the cells of a number of specimens thus fixed the inclusions were preserved as deeply staining discrete bodies, thus further substantiating the observation of Guilliermond (1908) that the bodies are aleurone grains. Undoubtedly they are not mitochondria. Since none of the fixatives used preserved the mitochondria, yet destroyed the aleurone grains, it cannot be stated definitely that mitochondria are present during the resting stage. That they are present, however, but masked by the aleurone grains can be assumed, since their occurrence can be demonstrated in the wheat

grain before the resting stage while the grain is still developing on the wheat plant.²

During the initial hours of germination, while the grain is swelling, little change is noted in the epithelial cells. Numerous aleurone grains are still preserved intact. Mitochondria are still not discernible, although Guilliermond reported the presence of basophilic granules from the first hours of germination.

STAGE I.—*Vacuoles*.—After germination continues for twenty-four hours, at which time the radicle has usually just broken through the outer coverings of the grain, marked changes occur in the epithelial cells. In numerous cells, small round or irregularly shaped vacuoles dispersed throughout the cytoplasm are evident, many of which enclose one or several small darkly staining granules (fig. 3). Guilliermond demonstrated the formation of vacuoles through the swelling and dissolution of the aleurone grains. The dark granules in the vacuoles at this stage are probably the fixation images of the precipitated remains of the aleurone grain protein. In some specimens at this stage a number of cells still retain intact numerous aleurone grains.

***Plastids and mitochondria*.**—The most striking difference brought about after twenty-four hours germination is the presence of numerous inclusions which are preserved by the mitochondrial fixatives. At this and the following stage, two types of inclusion are discernible. Representing the first type are many large, usually rod-shaped bodies dispersed throughout the cytoplasm (fig. 4). Often these inclusions are knobbed structures. Rather than rod-shaped, they sometimes appear rounded off and shortened (fig. 4). Frequently they contain round, optically clear vesicles which are often present at the end of the rod (fig. 4). At a later stage (IV), to be referred to, the vesicles in these inclusions can be identified as starch grains by means of a polarizing microscope. Thus these bodies are plastid primordia or, if starch is present, plastids. The presence of such structures should be expected, for Guilliermond (1908) and Toole (1924) reported the presence of starch in the epithelial cells during germination.

Representing the second type of inclusion present at this stage is a profusion of minute granular mitochondria (fig. 4). These bodies which are dispersed throughout the cytoplasm were never observed to develop into plastids.

Neither of these two types of inclusion shows any orientation which would suggest its passage through the end wall of the cell as reported for mitochondria in the epithelial cells by Horning and Petrie (1927). Instead they are present in equal numbers in all parts of the cytoplasm.

All of the above mentioned inclusions retain the stain deeply after fixation in modified Erliki's-pyridine mixture. They stain only lightly when Bensley's solution is used; moreover, the plastid primordia and

plastids are much contracted after fixation in this mixture (fig. 16).

STAGE II.—*Vacuoles*.—As germination continues and the shoots reach a length of 4 to 5 mm., the number of small vacuoles increases. In the majority of cells larger vacuoles appear, particularly in the basal part of the cell, due probably to the swelling and coalescing of smaller ones, as the number of vacuoles per cell decreases (fig. 5, 6). In the distal part of the cells the vacuoles, without undergoing any great increase in size, assume a tortuous, canalicular form, many of which are orientated parallel to the longitudinal axis of the cell (fig. 5, 6). In general, then, the cytoplasm appears more dense in the distal part of the cell than in the basal part. As the cytoplasm in these cells has been observed in rapid cyclosis, perhaps the shape of the vacuoles indicates only the condition of cytoplasmic movement at the moment of fixation and not some secretory function of the vacuole. The precipitated remains of the aleurone grains are still evident in some cells (fig. 5, 6).

As previously indicated in stage I, there is no marked change in the mitochondrial or plastid complex at this stage (fig. 4).

STAGE III.—*Vacuoles*.—As the shoots increase from 5 to 15 mm. in length, the cells become more vacuolate, large vacuoles forming in both the basal and distal parts of the cell as a result of further increase in size and coalescence. Often a single large vacuole, surrounded by a thin layer of peripheral cytoplasm, is formed in the extreme basal region. This vacuole, in the fixed cell, is usually empty (fig. 7) or contains, at most, a few strands of precipitated vacuolar material. While many specimens at this stage contain numerous cells with the empty basal vacuole, its presence is more characteristic of the next and later stages. In the enlarged vacuoles in the distal region there is contained a vacuolar material which is preserved usually as a lightly staining, homogeneous mass (fig. 7). This substance usually completely fills the vacuole so that often it cannot be delineated readily from the surrounding cytoplasm, especially after fixation in modified Erliki's-formalin or Bensley's mixture. While all the fixatives used preserve the vacuolar contents, in many specimens, particularly after fixation in chromium sulfate-formalin mixture, the vacuolar material is precipitated in irregular clumps (fig. 8), although an occasional cell in the same section will have vacuoles containing the homogeneous precipitate (fig. 9). In some cells, the basal vacuoles also contain the precipitated material (fig. 8). In vacuoles of many cells, usually one or occasionally two or three darkly staining, spherical granules are also preserved. They either occur free in the vacuole (fig. 12, 13) or are embedded in the non-staining vacuolar material, if both are present in the same vacuole (fig. 23, 25). Although preserved by all the fixatives here used, they are particularly evident after fixation in modified Erliki's-formalin mixture. Guilliermond (1908) reported the retention, in the earlier stages of germination, of some of the aleurone grain protein which

² No attempt was made to differentiate plastid primordia from non-plastid-forming mitochondria before germination had begun.

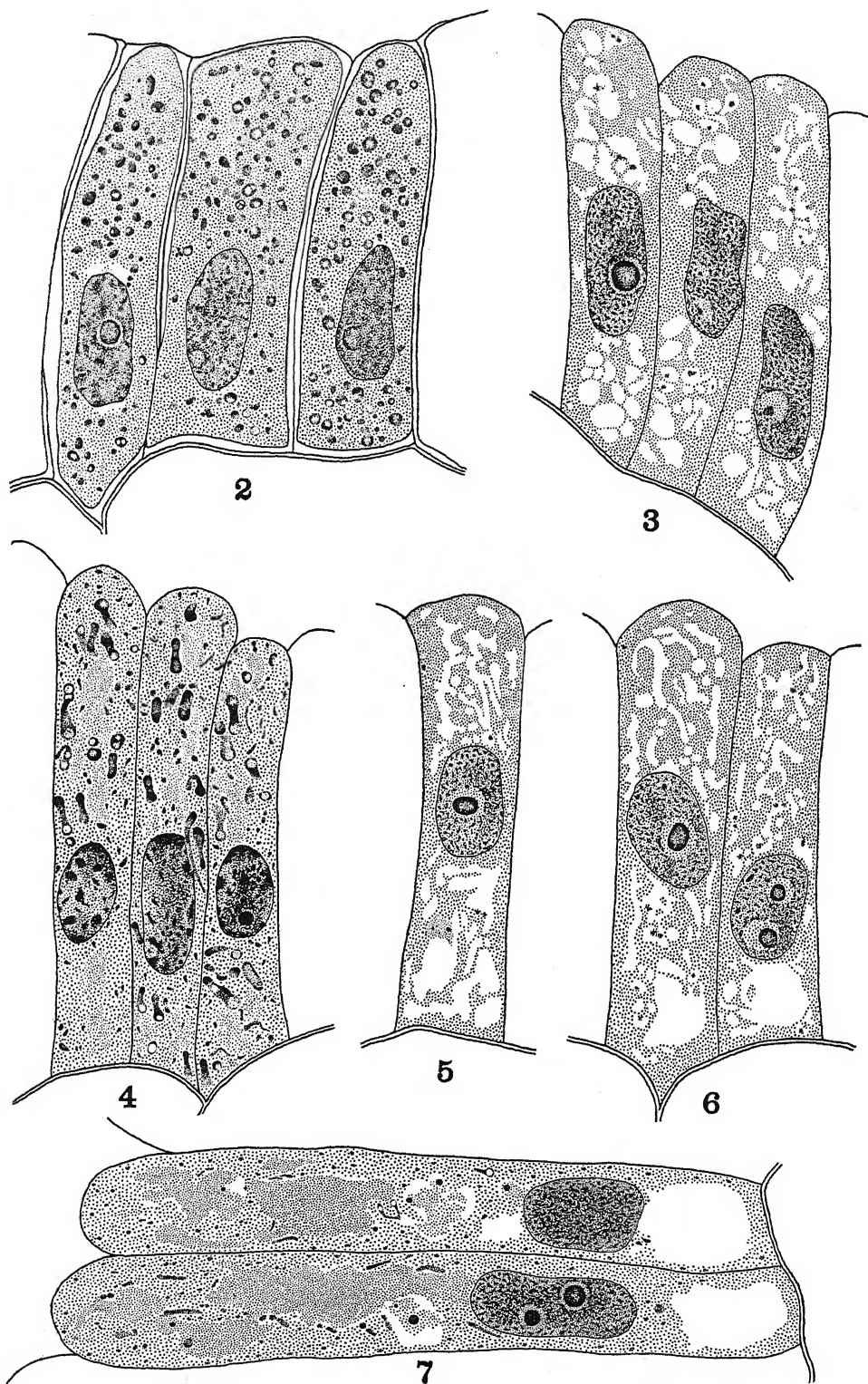


Fig. 2-7.—Fig. 2. Longitudinal section of epithelial cells in resting grain of *Triticum* fixed in modified Erliki's-formalin showing numerous small aleurone grains dispersed in the cytoplasm.—Fig. 3. Epithelial cells in germinating grain of *Triticum*. The radicle is just beginning to break through the outer grain covering. Modified Erliki's-formalin. Numerous small vacuoles formed from the dissolution of aleurone grains are evident. Some vacuoles still contain remnants of undissolved aleurone grain protein.—Fig. 4. *Secale*. Shoot 5 mm. Modified Erliki's-pyridine. Numerous large,

fused as the vacuoles coalesced. That these granules are of aleurone grain origin is doubtful, since they persist throughout the germination process and, at times, appear somewhat enlarged (fig. 10, 23, 25, 27, 31).

In a few specimens, fixation, particularly in modified Erliki's-formalin mixture, renders the cytoplasm and vacuolar material so homogeneous that the cell appears non-vacuolate except for the empty basal vacuole if it is present. That the distal region is vacuolate, however, can be concluded, since there is often present the above described, deeply staining, vacuolar granule. In such cases, the granule appears to be embedded in undifferentiated cytoplasm. Since so many other specimens show it to be contained in the vacuole, it can be concluded that the granule in this case is embedded in homogeneous vacuolar material which cannot be distinguished from the surrounding cytoplasm.

The mitochondrial and plastid elements dispersed in the cytoplasm retain the same general characteristics they displayed in stages I and II. In addition to the forms of plastids previously described, there are present occasionally small clusters of non-staining spheres held together in a thin, deeply staining matrix (fig. 11). The non-staining spheres are evidently starch grains.

Mitochondrial grouping.—In a number of cells at this stage, there is discerned, usually distad to the nucleus, a peculiar solitary grouping of large mitochondrial bodies. The attenuated elements extend in all directions from the center of a lightly staining, homogeneous mass and terminate in swollen head portions (fig. 11). The chance grouping of freely dispersed plastid primordia often renders identification of this structure difficult during this stage. However, in a number of cells, it is differentiated sufficiently from the dispersed plastid primordia to make identification definite. The difficulty in differentiating the structure from the plastid primordia occurring free in the cytoplasm may explain the failure to observe it in earlier stages. The grouping, to be more fully described, is better delineated in the following stage.

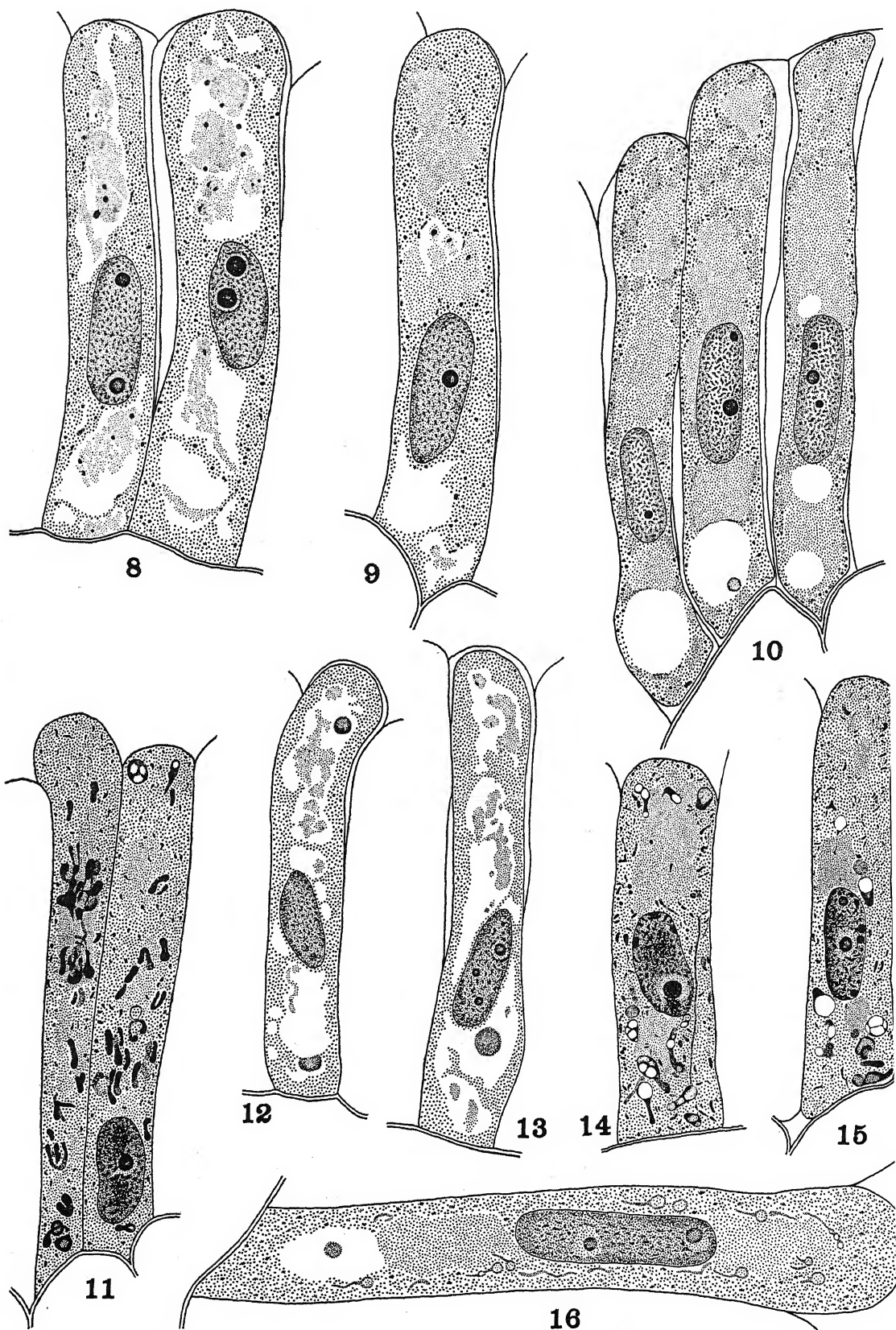
In many specimens, the cells have lengthened considerably during this stage and lateral separation, particularly of those cells in the anterior region of the layer, is evident.

Stage IV.—Vacuoles.—As the shoot increases in length from 15 to 30 mm., the various cytoplasmic inclusions become more distinct. Increased vacuolization characterizes the majority of cells. If the nucleus is basal, a vacuole extends from the nucleus to the distal end of the cell; if central, vacuoles are present in both the basal and distal regions (fig. 10, 16). The homogeneous vacuolar precipitate, de-

scribed in stage III, is present in the cells of practically all specimens as a lightly staining mass which completely fills the vacuole (fig. 10, 16). This material is preserved by all the cytoplasmic fixatives used. While in any one specimen this precipitate characterizes the majority of cells, yet some cells contain practically empty vacuoles, housing at most a few irregular clumps of vacuolar material. As noted in stage III, many cells contain an empty basal vacuole, especially towards the end of this stage. In such cells, the homogeneous vacuolar precipitate often extends to the very limits of the clear vacuole. In many cases the granular cytoplasm is not visible between the two areas, so that they appear contiguous (fig. 16). In some cells the presence of a thin membrane is discernible (fig. 10, middle cell). At other times a definite zone of granular cytoplasm separates the two regions. Occasionally the homogeneous material extends throughout the entire length of the cell, even into the basal region (fig. 10). Frequently the homogeneous area is interrupted by the presence of a small, clear vacuole (fig. 10, 21) in which the spherical granule, noted in stage III, is often retained. Guilliermond (1908) reported the presence of a substance which stained in a diffuse manner in the vacuoles in the distal part of the cells from the fifth day until the end of germination. After an examination of his figures, it is to be concluded that the diffusely staining vacuolar material and the homogeneously precipitated material here reported are the same substance. As noted by Guilliermond (1908), this material persists until the end of germination, at which time the vacuoles appear empty. As indicated in stage III, a few specimens, notably those fixed in chromic sulfate-formalin, disclose the presence of only a small number of cells containing the homogeneous precipitate; in the majority of cells, the vacuoles are open, elongated cavities which stretch throughout the length of the cell and contain the precipitated vacuolar material in irregular clumps (fig. 12, 13). Other specimens fixed in the same killing fluids contain the homogeneous material in the majority of cells (fig. 10). The homogeneous material should be regarded as vacuolar and not as a non-granular portion of the cytoplasm, for the region occupied by this material is clearly vacuolar in earlier stages (fig. 3, 5, 6), and even in this stage it appears as a vacuole in certain cells, particularly after fixation in chromic sulfate-formalin. Some evidence to the contrary should be noted, however. In some instances, the material itself seems to contain vacuoles which appear empty when preserved by the usual cytoplasmic fixation (fig. 10, 21).

Mitochondria and plastids.—As in the previous stage, a profusion of the minute granular mitochondria is dispersed throughout the cytoplasm (fig. 14—

deeply staining plastid primordia are evident. The vesiculated forms are plastids. Small granular mitochondria are dispersed throughout the cytoplasm.—Fig. 5-6. *Secale*. Shoot 5 mm. Modified Erliki's-formalin. In the basal part of the cell, vacuoles are enlarging and coalescing. Note the presence of canalicular vacuoles in the distal part. Aleurone grain protein remnants are still evident.—Fig. 7. *Triticum*. Shoot 11 mm. Modified Erliki's-formalin. Enlarged vacuoles in the distal region of the cell are filled with a homogeneous precipitate. Large, empty basal vacuoles are evident. Note lightly staining plastid primordia and plastids. All figures $\times 1,550$.



19). Occasionally small filamentous mitochondria are present among the granular forms (fig. 17, 18). The plastid complex, in general, appears as previously described. The plastid primordia are usually present in the form of homogeneous rods (fig. 17, 19, 20, 21). The plastid assumes a variety of forms, frequently being preserved as a rod containing one or several small, optically clear vesicles either at the end of the rod or strung along its length (fig. 14-16, 18-19). Present in many cells are dense clusters of small vesicles embedded in a deeply staining matrix noted in stage III (fig. 15). In some cells, these bodies appear as a single, enlarged, spherical vesicle with a deeply staining cap on one side or a thin sheath stretched around its periphery (fig. 15). Occasionally the cap is elongated so that the whole structure has the appearance of a tennis racquet (fig. 14). The larger of these optically clear vesicles are readily demonstrated, by means of a polarizing microscope, to be starch grains. The smaller vesicles could not be so tested but it is highly probable that they are starch grains.

Mitochondrial grouping.—The previously mentioned (stage III) solitary grouping of mitochondria³ is now readily discerned (fig. 17, 18, 20, 22, 24). The whole structure appears as a cluster of attenuated elements distinctly different in form from either the granular mitochondria or the plastid primordia present in the cells. Each element, of which there are a large but varying number in the cluster, is composed of a darkly staining, swollen head portion which tapers rapidly into an elongated, filamentous tail. The end of the tail is beyond the visibility of the microscope. The elements are usually so grouped that the tail portions arise in a lightly staining, optically homogeneous area (fig. 17, 18, 20, 24). The swollen head portions thus extend in all directions. Only rarely do any of the head portions appear vesiculated. Often the filamentous portions, while extending outward, are twisted and curved (fig. 17, 18). The elements retain the stain intensely after fixation in modified Erliki's-pyridine (fig. 17, 18, 20); it is on the basis of their preservation in this solution that they are here regarded as mitochondrial in nature. They stain only lightly after fixation in Bensley's mixture, as do the other cytoplasmic ele-

³ The labeling of these bodies as mitochondria is purely arbitrary. The term as here used merely indicates that they are preserved by those fluids which fix mitochondria and are dissolved by the fixatives which destroy mitochondria.

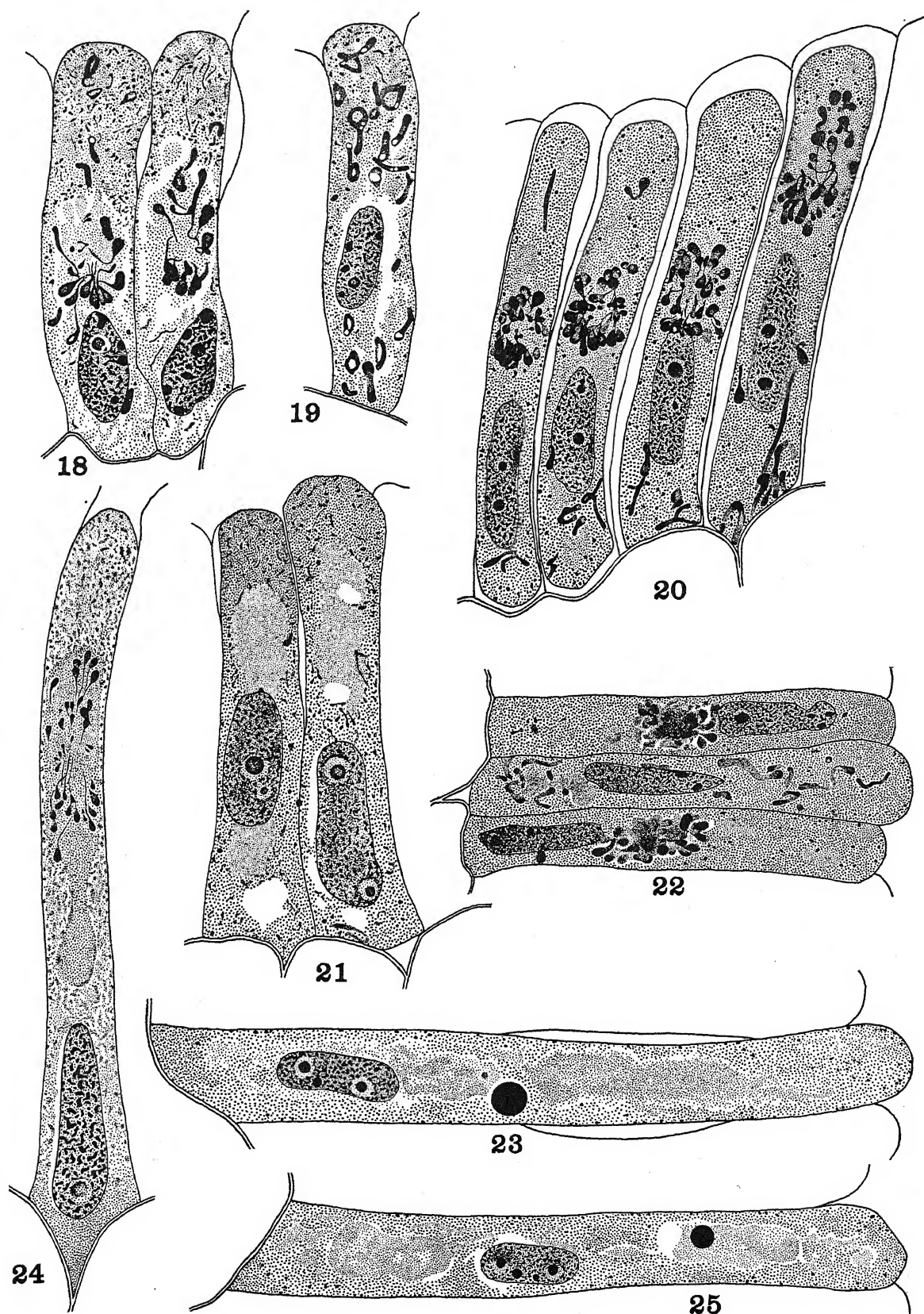
Fig. 8-16.—Fig. 8-9. *Triticum*. Shoot 11 mm. Chromium sulfate-formalin.—Fig. 8. Enlarged distal and basal vacuoles contain irregularly precipitated clumps of vacuolar material.—Fig. 9. Cell from the same section as cells in figure 8. The distal vacuoles contain the homogeneous precipitate. Note the presence of the empty basal vacuole.—Fig. 10. *Triticum*. Shoot 30 mm. Chromium sulfate-formalin. Large vacuoles, most of which are filled with the homogeneous precipitate, extend throughout the cells. Note the empty basal vacuoles. Small empty vacuoles are present in the homogeneous vacuolar precipitate in the cell on the right.—Fig. 11. *Triticum*. Shoot 12 mm. Modified Erliki's-pyridine. The mitochondrial grouping is evident in the left cell. The homogeneous area in which it is contained is not readily delineated. Note several plastids in addition to numerous plastid primordia and granular mitochondria.—Fig. 12-13. *Secale*. Shoot 29 mm. Chromium sulfate-formalin. Large extended vacuoles contain irregularly precipitated clumps of vacuolar material. Compare with figure 10. Note large vacuolar granules.—Fig. 14-15. *Secale*. Shoot 28 mm. Modified Erliki's-pyridine. Various shaped plastids containing starch grains are present. Note granular mitochondria.—Fig. 16. *Triticum*. Shoot 20 mm. Bensley's. Large vacuoles containing the homogeneous precipitate and the empty basal vacuole are present. Note lightly staining plastid primordia and plastids. All figures $\times 1,550$.

ments in the cell, and the head portions are not so swollen as they are after fixation in modified Erliki's-pyridine (fig. 24). In cells killed in modified Erliki's-formalin, the structure is only vaguely discernible, staining slightly more deeply than its cytoplasmic



Fig. 17. *Secale*. Shoot 25 mm. Modified Erliki's-pyridine. Each cell contains the deeply staining, solitary mitochondrial grouping confined to a homogeneously precipitated area which appears vacuolar. Note plastid primordia, granular mitochondria and several small mitochondrial filaments. $\times 2,300$.

surroundings (fig. 22). It is not discernible after chromic sulfate-formalin fixation. The structure is not present in the granular cytoplasm; it is confined to a lightly staining homogeneous region the fixation image of which appears very similar to the previously described homogeneous material precipitated within the vacuole (fig. 17, 18, 24). This region appears, in many cases, to have been pulled away from the surrounding granular cytoplasm so that a thin,



peripheral, clear zone is discerned after fixation in both modified Erliki's-pyridine and modified Erliki's-formalin (fig. 17, 18, 22). In some cells fixed in the latter solution the central portion of this region stains more deeply (fig. 22). When Bensley's fixative is used, this region is so fixed that it is in intimate contact with the granular cytoplasm. When thus fixed it is extremely difficult to delineate the region accurately (fig. 24). This is also true of the above mentioned precipitated vacuolar material (fig. 16.) Often the homogeneous region occupies little more area than the mitochondrial grouping contained within it (fig. 17). In other cases, the region is more expanded, extending beyond the grouping and appearing not unlike the previously described extended vacuole containing the homogeneous precipitate (fig. 24). It is difficult at times to determine whether or not the elements of the grouping extend beyond the homogeneous region into the surrounding cytoplasm. Usually, however, they appear to be confined within the region, although some of the head portions often extend to its very limits. In cells in which the grouping is more expanded, the greater number of elements extend necessarily toward the basal and distal ends of the cell because of the elongated cell shape (fig. 24). While in many cells the grouping is present just distad to the nucleus, its position varies from basal to distal. It appears only occasionally, however, in the region basal to the nucleus (fig. 22). The specimens varied in the number of cells in which the grouping could be demonstrated: in some, the greater number of cells contain the structure; in other, it could be delineated only occasionally. Since the fixation image of the homogeneous area housing the grouping appears very similar to the vacuolar homogeneous precipitate and since it is a definite zone set off from the granular cytoplasm, often being pulled away from it in fixation, it would appear that this region is vacuolar in nature. If this conclusion is correct, such a structure would indeed be unique in that it contains elements of mitochondrial nature which, moreover, assume a definite orientation, a condition in itself unique. It should be emphasized again, however, that future investigation may make it more convenient to consider this structure a highly specialized portion of the cytoplasm rather than a discrete vacuole.

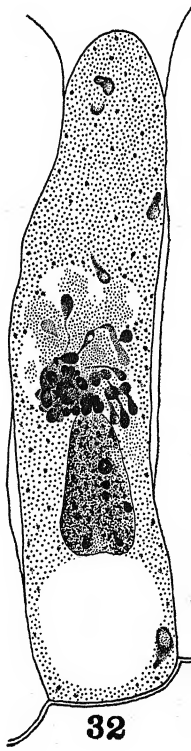
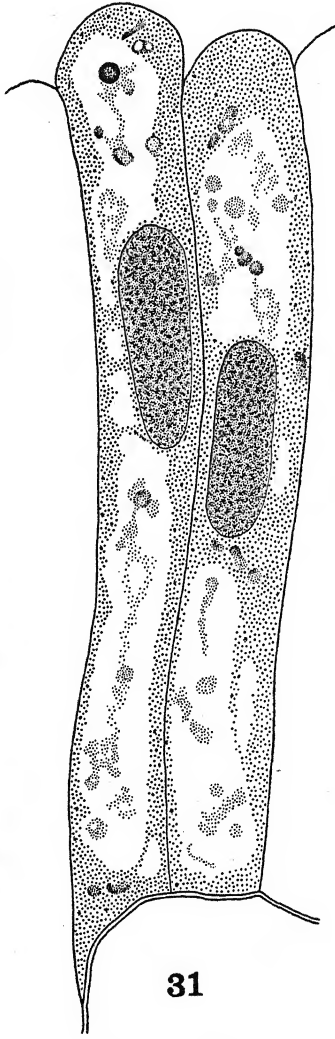
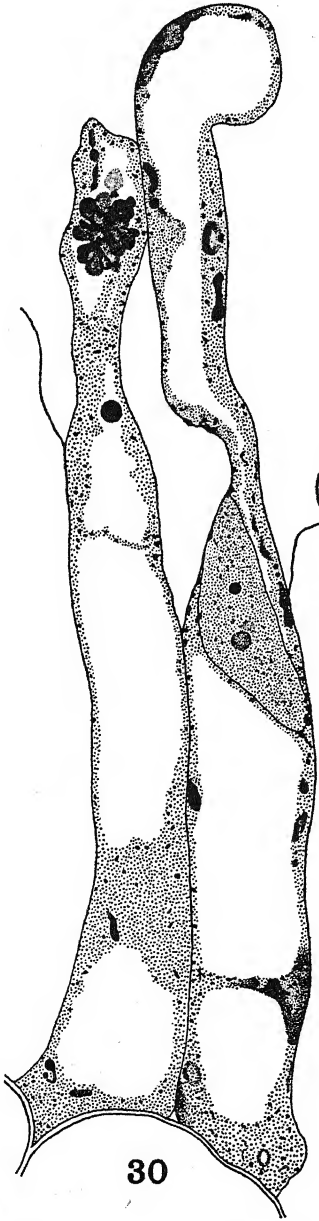
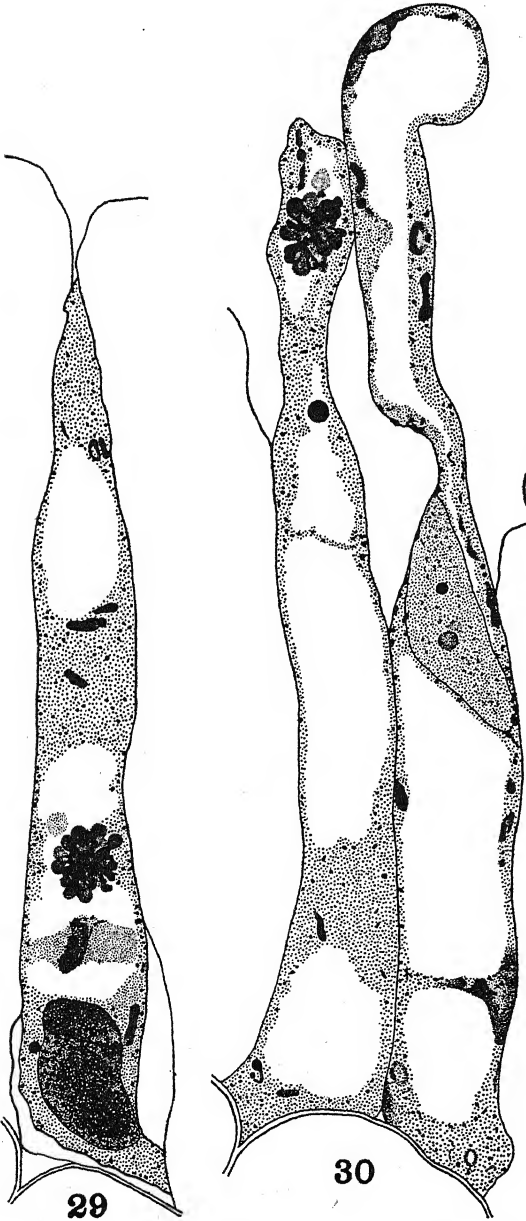
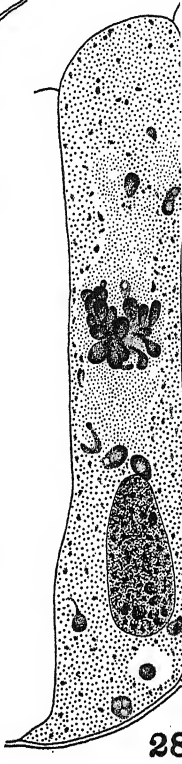
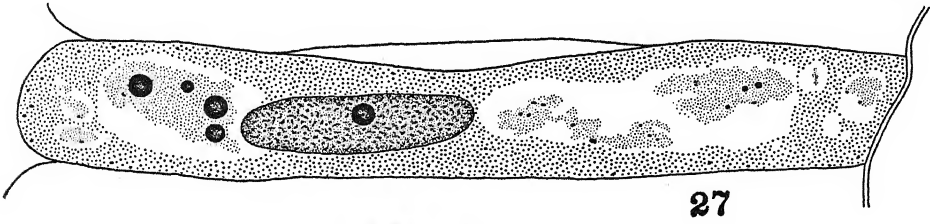
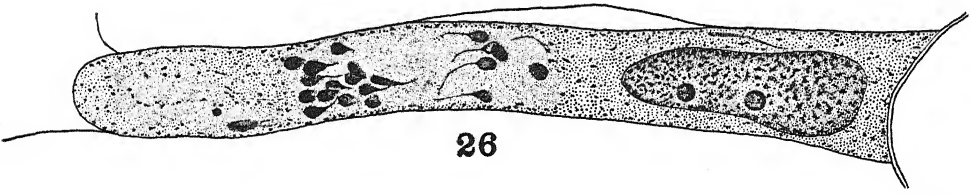
As is evident from the accompanying figures, the cells of all specimens have not attained equal length at this period of germination. In some, the cells have reached a maximum length, and the lateral separa-

tion is pronounced; in others, the cells are apparently lengthening. There is a clear correlation between the length of the cells and their position in the epithelium: the cells are longer in the anterior region and shorter in the posterior. The gradation in length is regular. This difference in cell length persists until the end of germination. It is also in the anterior region that the cells attain greatest lateral separation, at the same time becoming highly irregular in shape, particularly in the final stages of germination. In some specimens this occurs to a lesser degree in the cells in the posterior region. In the majority of specimens, little or no separation or distortion characterizes the cells in the central region of the layer.

STAGE V.—*Vacuoles*.—As germination continues, there is little change in the epithelial cells over an extended period, and it is not until the endosperm is almost depleted that more pronounced alterations occur. Thus, even when the shoots have reached a length of 7 or 8 cm., the condition of the cells in many specimens varies little from that in the last described stage. The usually empty, basal vacuole is present in the majority of cells, although even at this stage it is of infrequent occurrence in some specimens. Its presence was also reported by Guilliermond (1908) as characteristic of the later stages of germination. In some of these vacuoles an irregular precipitate occurs. The majority of cells still show the extended areas of lightly staining, homogeneous vacuolar precipitate, which, as previously noted, is often contiguous with the empty, basal vacuole or, when the latter is not present, frequently extends into the basal region of the cell (fig. 25). When the nucleus occurs in the distal region of the cell, the homogeneous area often extends from the proximal end of the nucleus to the empty basal vacuole. In a few cases, when the empty vacuole is not completely basal, the homogeneous area borders on both its distal and proximal sides. In some specimens the homogeneous material appears to have been pulled away from the vacuolar membrane, probably as the result of fixation (fig. 23, 25).

In a number of cells in the anterior region of the epithelial layer of many specimens, the vacuoles do not contain the characteristic homogeneous precipitate but instead contain irregular clumps of precipitated material. In a few specimens this condition appears in cells dispersed throughout the whole layer. In other grains, particularly toward the end of this stage, this condition is characteristic of the majority of cells (fig. 27), and only an occasional cell

Fig. 18-25.—Fig. 18-19. *Secale*. Shoot 25 mm. Modified Erliki's-pyridine. Cells from the same specimen as the cells illustrated in figure 17.—Fig. 18. Note the varying form of the mitochondrial grouping when compared with figure 17.—Fig. 19. The mitochondrial grouping is not present. Note the variously shaped plastids and the granular mitochondria.—Fig. 20. *Triticum*. Shoot 25 mm. Modified Erliki's-pyridine. The cytoplasmic inclusions are similar to those illustrated in figures 17-18, although the homogeneous area containing the mitochondrial grouping is not as sharply delineated.—Fig. 24. *Triticum*. Shoot 20 mm. Bensley's. The lightly staining mitochondrial grouping is evident. The cell is from the same specimen as the cell illustrated in figure 16.—Fig. 21. *Triticum*. Shoot 29 mm. Bensley's. Note the small empty vacuoles contained in the homogeneous vacuolar precipitate.—Fig. 22. *Secale*. Shoot 25 mm. Modified Erliki's-formalin. Two cells contain the mitochondrial grouping. Note the deeply staining center of the grouping. Lightly staining plastid primordia are present in the middle cell.—Fig. 23 and 25. *Secale*. Shoot 60 mm. Modified Erliki's-formalin. Extending throughout the cell are large vacuoles containing the homogeneous precipitate. Note the large, deeply staining vacuolar granule embedded in the homogeneous material. All figures $\times 1,550$.



displays the homogeneous precipitate. Since the vacuoles are usually empty at the end of germination this condition must indicate a gradual depletion of the vacuolar material that is precipitated in fixation.

There is no marked change in either the plastid or mitochondrial complex at this stage. In many cells the plastids contain enlarged starch grains; in others the typical plastid primordia are retained. The number of granular mitochondria has not decreased when this stage of germination is reached. There is little change in the peculiar mitochondrial grouping, either in form or position (fig. 26).

STAGE VI.—Vacuoles.—At the end of germination, as the endosperm becomes emptied, the shoots attain a length of from 12 to 15 cm. At this stage the cells are highly vacuolate, the vacuoles appearing empty or at most containing a few irregular clumps of precipitated material (fig. 31). In many cells only a thin peripheral layer of cytoplasm is present so that large, empty vacuoles occur on either side of the nucleus, a condition also observed by Guilliermond (1908). In cells which are evidently beginning to disintegrate, the nucleus stains irregularly and in some specimens becomes rounded, while in others it assumes an irregular form (fig. 29, 30). In other specimens the contents of some of the cells appear to have dissolved so that little is left except the cell wall.

Plastids and mitochondria.—The plastid and mitochondrial elements evidently persist until the cells begin to disintegrate. A number of the large plastid primordia are still present in many cells (fig. 29, 30). A definite plastid with contained starch grains is discernible in rare instances. The granular mitochondria are still evident (fig. 29, 30). Horning and Petrie (1927) reported the disappearance of the migratory mitochondria as germination draws to a close, a condition they identified indirectly with cessation of secretory activity. It is to be noted, however, that on the completion of the germination process the epithelial cells begin to disintegrate, and it is quite possible that to this condition is due the disappearance of cytoplasmic inclusions.

Mitochondrial grouping.—The mitochondrial grouping can still be demonstrated in some cells at this stage. It is now greatly contracted, appearing as a darkly staining rosette. From the usually deeply staining, undifferentiated center, the head portions project in all directions (fig. 29, 30). The elongated tails have evidently contracted to draw the head portions together. The homogeneous area in which the grouping is contained in earlier stages is here not

discernible. While the exact position of the dense grouping in relation to the vacuole is at times difficult to discern, there is evidence to indicate that it is present within the vacuole. Usually it appears to be confined within an area devoid of cytoplasm which evidently is a vacuole. While a portion of the rosette may extend to the cytoplasm, the greater part appears free in the vacuole (fig. 29, 30). It is to be noted that in a specimen of comparable shoot length in which the epithelial cells are not so advanced as here described, the grouping in most cells appears as in earlier stages and is contained within the homogeneous area. In a few cells, however, the grouping, although still within the homogeneous area, assumes the contracted form characteristic of the final stage (fig. 28). In still other cells, while the greater number of elements are contracted to form a dense rosette, a few retain the elongated form and project a distance beyond the main grouping, but are still contained within the homogeneous area (fig. 32).

With endosperm depletion, the secreting and absorbing activity of the epithelial cells comes to an end. This cessation of function is accompanied by an obvious disintegration of the specialized structure which characterizes the cells. The cells themselves soon die and disintegrate.

Discussion.—While it is evident that the epithelial cells of the scutellum are gland cells, the changes occurring during activity and the presence of specific structures must be interpreted in terms of two functions, since the cells absorb the digested endosperm material as well as secrete enzymes.

Vacuoles.—Neither the structure nor the position of the vacuoles in the epithelial cells give positive evidence that they play a rôle either in secretion or absorption. The nature of the precipitated homogeneous material present within the vacuoles is as yet undetermined. It is highly probable that some of the soluble carbohydrates absorbed from the endosperm collect in the vacuoles, but such a substance would not be precipitated by the type of fixation here used. The fact that the material is precipitated by the mitochondrial fixatives indicates perhaps that it is of a protein nature. Such a supposition suggests several possibilities as to its origin. That it might be a fixation image of a fine dispersion of a colloiddally dispersed protein which remains within the vacuoles after the dissolution of the aleurone grains is extremely doubtful. Such a conclusion, moreover, would not be supported by the earlier observations of Guilliermond (1908). Another possibility is that the precipitated substances is actually an enzyme, pos-

Fig. 26-32.—Fig. 26. *Triticum*. Shoot 95 mm. Bensley's. The mitochondrial grouping contained within the homogeneously precipitated area is present.—Fig. 27. *Triticum*. Shoot 80 mm. Modified Erliki's-formalin. Large vacuoles extending throughout the cell contain irregularly precipitated clumps of vacuolar material. Note the vacuolar granules.—Fig. 28 and 32. *Triticum*. Shoot 110 mm. Modified Erliki's-pyridine. A solitary contracted mitochondrial grouping is contained within the homogeneous area. In figure 32 some of the elements of the contracted grouping are still extended, their form appearing very similar to that of earlier stages.—Fig. 29-30. *Triticum*. Shoot 150 mm. Modified Erliki's-pyridine. In two cells the mitochondrial grouping is greatly contracted and now no longer appears to be contained within the homogeneous area. The vacuoles are empty. Note plastid primordia and granular mitochondria.—Fig. 31. *Triticum*. Shoot 150 mm. Modified Erliki's-formalin. The greatly extended vacuoles contain only a few irregularly precipitated clumps of vacuolar material. Note vacuolar granules. All figures $\times 1,550$.

sibly a protein, produced by the epithelial cells and collected in the vacuoles before it is discharged, in some unknown manner, into the endosperm. As previously noted, this homogeneous material is concentrated particularly in the distal region of the cell and, as the endosperm approaches complete depletion, it tends to disappear.

The position of the empty, basal vacuole suggests that it functions in absorption. The soluble carbohydrate material absorbed from the endosperm by the epithelial cell may collect temporarily in this vacuole from which it is eventually carried away through the scutellar parenchyma to the growing plant.

Plastids and mitochondria.—Of the three types of inclusions preserved by the mitochondrial fixatives in the epithelial cells during germination, a definite function can be assigned to only one. The large, usually rod-shaped bodies are plastid primordia. These inclusions readily develop into plastids as optically clear vesicles which can be identified as starch grains form within them. In typical meristematic cells, two categories of mitochondria have been recognized, the one composed of plastid primordia, the other including the forms not developing into plastids. Satisfactory criteria for differentiating the two categories are still unavailable. In the epithelial cells, however, plastid primordia can be differentiated on the basis of size and shape from non-plastid-forming mitochondria. It is concluded that all of the large, usually rod-shaped inclusions are plastid primordia, since in addition to the fact that it is only from this type of inclusion that the plastids arise, there is the further evidence presented in some cells in which practically all of these bodies have developed into plastids (fig. 14, 19). The presence of such structures can be interpreted in terms of absorption only, for they function as a temporary storage place for starch which is later reabsorbed as germination draws to a close.

No definite function can be assigned to the numerous minute granular mitochondria present throughout germination. It is possible that they belong in the same category as the non-plastid-forming types of mitochondria found in meristematic cells and have arisen from such types as the development of the epithelial cells proceeds from undifferentiated meristematic tissue. The evidence at hand, other than their existence in large numbers, does not warrant an assumption that they here acquire a function connected with the specialized activity of the epithelial cells.

Mitochondrial grouping.—The grouping of the large mitochondrial elements in the epithelial cells is unique. The elements themselves are peculiar in that, when fixed, they always assume a form in which a definite head and tail can be differentiated. In addition, the elements are definitely oriented in a characteristic group. They are not present in the granular cytoplasm but are contained within a homogeneous region which the evidence at hand indicates to be a highly specialized type of vacuole. The structure as here described has been observed only in fixed cells,

so what has actually been seen is a fixation image of a cell inclusion that may have a somewhat different form in the living cell. However, since several types of fixative preserve the structure with little change in form, its fixation image may approximate very closely its structure in the living cell. As far as is known, such a structure has not been previously reported as occurring in any other type of cell. The evidence at present does not warrant the assigning of a particular function to this structure; but the fact that it occurs only in specialized glandular cells perhaps indicates that it is associated in some way with secretory activity. Such an assumption must be made with caution, however, for in the past, the mere presence of a particular body within a cell has led many investigators to assign a specific function to the body without the support of critical evidence.

It must be noted that there is some indication of the presence of the mitochondrial grouping in the sub-epithelial layers of the parenchyma cells of the scutellum as well as the other types of inclusion and the homogeneous vacuolar material. A cytological investigation of these cells is in progress.

SUMMARY

The epithelial cells of the scutellum of *Triticum sativum* and *Secale cereale* are organs of secretion and absorption. The internal changes occurring in the cells during the course of their activity and the presence of specific structures are considered in terms of these two functions.

As germination begins, small vacuoles form in the cells through the swelling and dissolution of aleurone grains present in the resting stage. As germination progresses, these vacuoles enlarge and coalesce. In the large vacuoles thus formed, there appears a vacuolar material which is precipitated in fixation as a lightly staining homogeneous mass. This material, possibly protein in nature, disappears at the end of germination, at which time the cells are completely vacuolate and devoid of vacuolar material.

Plastid primordia, separated on the basis of size and shape from non-plastid-forming mitochondria, are present in the epithelial cells during germination. These bodies readily develop into plastids as starch grains form within them.

Present also in the epithelial cells are two types of non-plastid-forming mitochondria. The first is represented by a profusion of minute granular bodies of unknown function occurring free in the cytoplasm. The second type of mitochondria, so designated because it is preserved by certain fixatives, does not occur freely dispersed; instead, these elements, each composed of a filament and enlarged terminal portion, are localized in a characteristic solitary group. The filamentous portions project in all directions from the center of the group and terminate in swollen heads. The structure is not present in the granular cytoplasm but is contained within a homogeneous region indistinguishable from the precipitated homogeneous vacuolar material. While this region may be a highly specialized portion of the cytoplasm, the

evidence at hand indicates that it is vacuolar in nature. Relationship between the structure and secretion is suggested.

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THE RELATIONSHIP OF RANUNCULUS TO THE NORTH AMERICAN FLORAS¹

Lyman Benson

THE OBJECT of this paper is to present the broad outlines of the geographical distribution of *Ranunculus*, which have emerged as a result of monographing the group for North America north of Mexico (Benson, 1936, 1940, 1941-2) and as a result of collecting 11,000 plant specimens, including several hundred sheets of *Ranunculus*, in the field. Combined with an interpretation of the distribution of the genus is an attempt to classify the many vegetation-areas with which the genus is associated, and to point out the significance of *Ranunculus* in the definition of major floristic areas and in the determination of the possible origin and relationships of the North American complex of floras.

The distributional pattern of *Ranunculus* in North America is by no means obvious, since it is complicated by the fact that the genus includes at least three distinct phases or groups of species, as follows: (A) the widespread aquatic and palustrine types; (B) the relics of ancient floras; (C) the dominant phase of the genus.

A. THE WIDESPREAD AQUATIC AND PALUSTRINE TYPES.—Although some aquatic, palustrine, or sub-palustrine species and varieties are of restricted distribution and are characteristic of particular areas, most are widespread, and their ranges are not necessarily restricted by exactly the same set of factors which governs the occurrence of terrestrial species. The widespread species of *Ranunculus* are as fol-

lows: EURANUNCULUS. Sect. *CHRYSAN-
THE*. *R. Macounii*, *R. pensylvanicus*. Sect. *FLAM-
MULA*. *R. Flammula* vars. *ovalis* and *filiformis*.
Sect. *HECATONIA*. *R. sceleratus*, which may be
introduced, *R. sceleratus* var. *multifidus*, *R. Gmelinii*
var. *terrestris* (*R. Purshii*), *R. flabellaris* (*R. del-
phinifolius*). CYRTORHYNCHA. Sect. *HAL-
ODES*. *R. Cymbalaria* and its varieties. BATRA-
CHIUM. *R. aquatilis* var. *capillaceus*, *R. circinatus*.

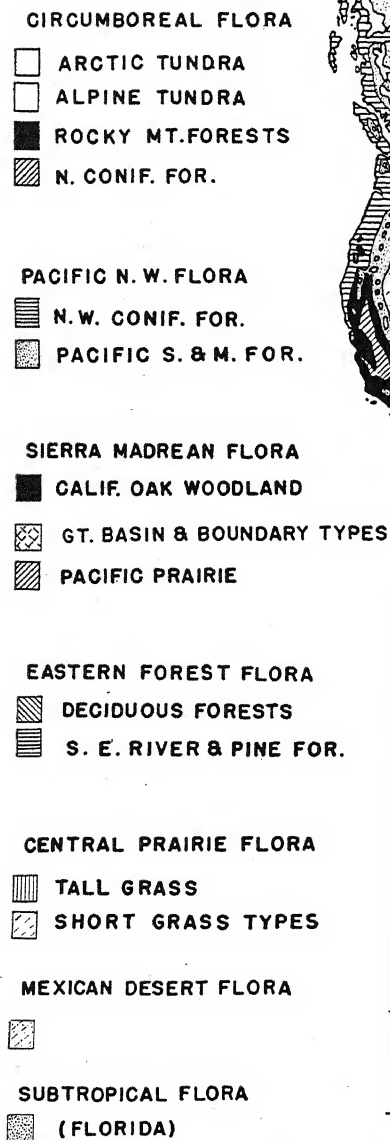
B. THE RELICS OF ANCIENT FLORAS.—Five of the seven native subgenera are small or monotypic and are represented by isolated species of disjunct distribution, as follows:

a. *Cyrtorhyncha*.—Eliminating the widespread palustrine types of the section *Halodes*, the subgenus is represented in North America by three monotypic sections, the species of which are discussed below: *Ranunculus ranunculinus* is an endemic of the frontal range of the Rocky Mountains in southeastern Wyoming and in Colorado. *R. Cool-
eyae* is known from coastal Alaska, from scattering mountain stations in coastal British Columbia and from a point at the southern end of the Olympic Mountains in Washington just southwest of the tip of the Kansan ice-sheet. *R. hystriculus* has a range in California almost coinciding with that of the Big Tree, *Sequoiadendron giganteum* or *Se-
quoia gigantea*, in the Sierra Nevada, and may share its antiquity. According to Asa Gray (1886, p. 365), *R. hystriculus* is approached in its dis-

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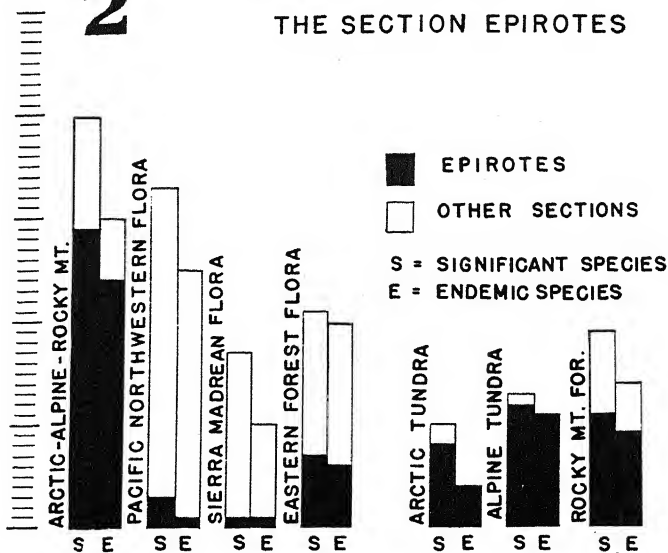
1

MAP SHOWING
THE NORTH AMERICAN FLORAS



2

DISTRIBUTION OF
THE SECTION EPIROTES



tinctive fruit characters by "certain New Zealand and Aucklandian Ranunculi." If these Antipodal species are as nearly related as Gray indicated, the distribution of the group is of a remarkably disjunct type, and it is almost certainly ancient. Each of the three species is definitely on the down-grade, depleted of bio-types, without adaptability, merely hanging on in favorable localities wherein it must have been long established.

b. *Oxygraphis*.—The single American species, *R. kamchaticus*, occurs in boreal and alpine Asia and on the Seward Peninsula of Alaska. This species and *R. Chamissonis* (subgenus *Crymodes* below) may be additional examples of plants which once occurred along an intercontinental land-bridge and which persisted on the American side of Behring Strait during the Pleistocene glaciations (Nelson, 1887; Adams, 1905, p. 58). (Northern Alaska was not glaciated in Pleistocene time.)

c. *Crymodes*.—This subgenus consists of two groups of species, as follows: (1) boreal and alpine types, including *R. glacialis* (Greenland, Iceland, and Europe), *R. Chamissonis* (Siberia, and north-western Alaska), and *R. Shaftoanus* (Afghanistan); (2) western North American semi-desert types, including *R. Andersonii* and *R. juniperinus* (Southwestern Coniferous Woodland or juniper-pinyon type and Northern Desert or sagebrush type in the Great Basin).

d. *Pallasiantha*.—*R. Pallasii* may be restricted to non-glaciated areas, since it occurs in northern Alaska and in scattering areas along the Arctic Ocean and at one station on the Arctic Archipelago (Polunin, 1940). (Parts of the Arctic shore and nearly all of the Archipelago remained unglaciated in Pleistocene times.) This species, which is not aggressive, is without close relatives, and lacks variability, may be the last surviving type of a more plastic population practically exterminated by glaciers. However, herbarium specimens are not abundant enough to indicate the exact distribution of Arctic species, and it is difficult to match up the data on all the labels with glaciated and unglaciated areas.

e. *Coptidium*.—*R. lapponicus* encircles the earth in boreal regions and in sub-boreal forests, but its distribution is not wholly continuous.

Although the species of the five subgenera discussed above show a disjunct distribution, their occurrence is in accord with the following outline. The greatest development of the group as a whole is in boreal and northern alpine regions or sub-boreal or subalpine forests of Eurasia and North America, and the species-groups with the most nearly continuous distribution occur in these areas. One group (that cited by Gray) is represented in New Zealand, and others occur as scattered endemic relics in western North America. It is possible that the discontinu-

ously distributed, unaggressive small subgenera represent relics of specialized branches of an older phase of the genus than is represented by the now more abundant and variable and frequently aggressive sections of the subgenus *Euranunculus*. If this is true, however, it is to be noted that the flowers of some and the fruits of all the surviving species have considerably more highly specialized features than those found in *Euranunculus* (cf. Benson, 1940, pl. I, and the keys to the subgenera and sections). The surviving types must represent only a small selection from a once highly variable and very extensive series of species, differentiated long ago.

Two other groups of relic-species indicate relationship of North American floras to those of other continents.

a. *Pacific Coast*.—As mentioned by Abrams (1929), many California plants are clearly related to Old World groups, and as shown by Fernald (1929) in the same series of papers, many of the species of Pacific North America are identical with or related to those of Western Europe, while the eastern North American Flora has elements in common with the eastern Asiatic flora. In the California Oak Woodland and in disjunct areas in Western Oregon and the Palouse Region in Washington and Idaho, *Ranunculus hebecarpus* is a common and characteristic species, and it is the only American representative of the section *Echinella* of the subgenus *Euranunculus*, a group strongly centering in Western and Central Europe and the Mediterranean Region. Furthermore, the Pacific Coast endemic, *R. Lobbii* (subgenus *Batrachium*), has no close American relatives, and, according to W. B. Drew (1936, p. 8), it is most closely related to the European species, *R. tripartitus*. As may be expected with an ancient relic-species, its distribution is discontinuous, since it occurs on Vancouver Island in British Columbia, at Corvallis in Oregon, and in the San Francisco Bay Region in California.

b. *Atlantic Coastal Plain*.—*Ranunculus Flammula*, a well-known European species, occurs in North America in southeastern Newfoundland, where (Fernald, 1917, p. 135) "... it is associated with many other typical western European species unknown elsewhere in North America. . ." Another European *Ranunculus*, *R. hederaceus*, occurs in the same area as well as on the floristically related (Fernald, 1911) Atlantic Coastal Plain in Chester County, Pennsylvania, and about Chesapeake Bay as well as (according to report) in South Carolina. *R. Ficaria* grows also in a few localities on the Coastal Plain, but it has been considered as introduced.

C. THE DOMINANT PHASE OF THE GENUS.—With the widespread aquatic and palustrine types and the ancient relic phase of the genus disposed of, the re-

Plate I.—Fig. 1-2. The North American Floras.—Fig. 1. Map showing the distribution of the North American Floras. Some subdivisions are combined as indicated in the legend.—Fig. 2. Bar diagram showing the relative numbers of species in the section *Epirotes* and other sections in the floras in which the genus is well represented. The section *Epirotes* dominates three subdivisions of the circumboreal flora, i.e., the Arctic Tundra, the Alpine Tundra, and the Rocky Mountain Forests, and elsewhere it is represented by only a few species all of which are aberrant.

maining species and varieties, which are practically all in the subgenus *Euranunculus* and which constitute the bulk of the species, may be segregated with ease into clear-cut geographical groups. These groups correspond to the following North American regional floras: the Circumboreal Flora, the Pacific Northwestern Flora, the Sierra Madrean Flora, and the Eastern Forest Flora. In addition to these four floras, three others occur in North America north of Mexico, but they will not be discussed in this paper, since *Ranunculus* is not represented in them or is represented by only a few terrestrial types. They are as follows: the Central Prairie Flora (tall grass and various short grass types), the Mexican Desert Flora (creosote-bush type), and the Subtropical Flora (mangrove; everglades).

1. *The circumboreal flora.*—For reasons to be given in the succeeding paragraphs, the following vegetation-types are included in the Circumboreal Flora: the Arctic Tundra, the Alpine Tundra, the Rocky Mountain Forests (subalpine and montane taken together), and the Northern Coniferous Forest.

The distinctness of the boreal flora is well-known, and, as has been pointed out by J. D. Hooker (1862) and others, it has had a nomadic existence, having followed the movements of glaciers, appearing always along their margins. In view of the northward and southward migrations of ice-sheets through the ages, it is not surprising that it is possible to trace the relationship of the *Ranunculi* and other plants growing in polar regions to those in the ecologically similar alpine type to the southward. However, it is surprising that in the case of *Ranunculus* relationship can be traced to the species occurring in the Rocky Mountain subalpine and montane forests but not to those in forests of apparently similar ecological type occurring elsewhere on the continent.

Although the species of *Ranunculus* occurring in the North American Arctic Tundra, Alpine Tundra, and Rocky Mountain subalpine and montane forests are for the most part mutually exclusive, their close relationship to one another is obvious, since they are dominated by the section *Epirotetes* of the subgenus *Euranunculus* and since the species of this section occurring elsewhere are few and aberrant (cf. solid areas in figure 2). In the region as a whole there are forty significant native species and varieties (excluding, as in all the rest of this paper below, very weakly represented plants and the types discussed under A and B above). Twenty-nine of these are in the section *Epirotetes*, and these include all but six of the thirty endemics. In the other floras, the section is represented by only three small, aberrant groups, as follows: *R. glaberrimus* and its varieties (Great Basin and northern Great Plains), *R. rhomboideus* (Central Prairie Flora), *R. abortivus*, *R. micranthus*, *R. allegheniensis*, *R. Harveyi*, and their varieties (largely in the Eastern Forest Flora).

The predominance of the section *Epirotetes* in the arctic-alpine-cordilleran area as a whole is also characteristic of each of the principal subdivisions

(solid areas in figure 2), except, as will be shown in the next paragraph, the Northern Coniferous Forest. In the Arctic Tundra eight of the ten species and all four endemics are in the section which includes, in the Alpine Tundra, twelve of the thirteen species and varieties and all eleven endemics, and in the Rocky Mountain Forests eleven of the nineteen species and varieties and nine of the fourteen endemics.

The failure of the section *Epirotetes* to become established in the Northern Coniferous Forest (spruce-fir type) in Eastern Canada and along the border of the eastern United States seems anomalous for the following reasons: the area seems to be ecologically ideal; the species of the section are dominant both in the arctic regions just to the north and in the Rocky Mountain Forests just to the southwest; many other species and species-groups are related to those in the Rocky Mountain Forests. An explanation of this failure of *Ranunculus* to reflect the relationship of the Northern Coniferous Forest to sub-boreal and subalpine forests throughout the Northern Hemisphere and particularly to the sub-alpine and montane forests in the Rocky Mountains may be attributed to the lack of aggressiveness of the section *Epirotetes*. The area occupied by the Northern spruce-fir forest is approximately that denuded by glaciers in the Pleistocene period, and, were the members of the section *Epirotetes* developing new types and actively spreading, they should be expected to have filled in this area immediately or at least to have invaded the borders. This sluggishness of the typical members of the section in the East is matched by similar inactivity in the West, for only one Alpine Tundra or Rocky Mountain Forest species (the alpine *R. Eschscholtzii*) has penetrated northward along the glaciated mountains, and only one of the boreal species (*R. pedatifidus*, which is really sub-boreal) is represented in the Western mountains by more than scattered relics, probably of pre-glacial times (and there are very few of these²). Of the twenty-two species of *Ranunculus* actually occurring in the Northern Coniferous Forest,³ nine are widespread aquatic and palustrine types eliminated from consideration, and the rest are obvious derivatives of groups in adjacent areas other than the Rocky Mountain Forests. These are confined to ranges adjoining their parents. Since the spruce-fir forest of the north has been filled in not by the expected derivatives of the section *Epirotetes* but by aggressive widespread types and, along its borders, by Alaskan and Eastern Forest neighbors, it is

² *R. hyperboreus* (sect. *Hecatonia*) has a relic-colony on Old Hollowtop near Pony, Montana, and *R. pygmaeus* (section *Epirotetes*) occurs in the Canadian Rockies and on a few peaks as far south as Colorado. A collection from Capitol Peak (Penland, 1512, cf. Penland, 1941, p. 13) may be *R. nivalis*.

³ The area is poor in endemic *Ranunculi*, a fact in harmony with the findings of Raup (1941), who in seven seasons in the remote Athabasca-Great Slave Lake region found only two new species, except in sand-dunes, a habitat-type often rich in endemic relics in this area (Adams, 1902, p. 310) but uninhabitable for *Ranunculus*.

necessary to depend largely upon other floristic elements to determine its relationship to the Circumboreal Flora.

Having correlated the *Ranunculus* floras of the boreal, alpine, and cordilleran forest regions with one another, and having advanced a possible explanation for lack of correlation of these *Ranunculi* with those of the floristically related Northern Coniferous Forest, it is well to examine samples of other elements in the flora to determine whether their distributional patterns are in agreement.

Although (as is true of *Ranunculus*) the pteridophytes occupying the other major floral areas are largely endemic⁴, those growing in the arctic-alpine areas and the forests of the northern part of the continent are chiefly widely-distributed Eurasian-North American species, some of which extend southward into the other floral regions. These widespread boreal northern-forest, and subalpine-forest species are almost the only ones occurring in the Rocky Mountain Forests, where there are not more than half a dozen characteristic or endemic species. It is to be noted that these same species flourish in the glaciated areas defaulted by the section *Epirotes*, thus strengthening the contention that, in large part, the Northern Coniferous Forest is a portion of the Circumboreal Flora.

The circumboreal origin of the bulk of the Rocky Mountain Forest flora receives a negative sort of support from the shortage of gymnosperm species, which possibly may be correlated with their almost complete absence, in the Arctic Tundra extending around the world. In the Rocky Mountain Forests there are only eight species or varieties which are endemic or essentially⁵ so, and (excluding two Mexican species growing near the Boundary) only fourteen altogether. A more positive support is gathered from two junipers (*Juniperus communis* and *J. Sabina*), which occur near timber-line around the Northern Hemisphere and which are well-developed in the Rocky Mountain Forests but not in either the Pacific Northwestern Flora or the Eastern Forest Flora.

2. *The Pacific Northwestern Flora*.—The following subdivisions are included in the Pacific Northwestern Flora: the Northwestern Coniferous

⁴ The Pacific Northwestern Flora includes about thirty-five endemic pteridophytes, the Sierra Madrean Flora, twenty or more, north of the boundary, and the Eastern Forest Flora, fifty or more.

⁵ *Pinus flexilis*, *P. aristata*, *P. ponderosa* vars. *scopulorum* and *arizonica*, *Picea pungens*, *Abies concolor* (excluding var. *Lowiana* of the Pacific Coast), *A. arizonica*, and *Juniperus scopulorum*. In the Pacific Northwestern Flora there are a total of thirty-three gymnosperms of which twenty-five are endemic (the eight non-endemic species being shared with the Rocky Mountain Forests). In the Sierra Madrean Flora there are twenty-four species north of the Border, all endemics. In the Eastern Forest Flora there are twenty-two endemic species and along the margins two widespread northern junipers. A number of Pacific Northwestern species reach the Rocky Mountain System in Montana and Idaho, but this area belongs floristically with the Pacific Northwest.

Forest⁶; the Pacific Subalpine and Montane Forests. The Palouse Prairie is included with some hesitation because of its affinity in some degree with other grasslands and because, at least in so far as *Ranunculus* is concerned, it is merely a meeting ground for species of adjoining areas. On the map (fig. 1) it is not differentiated from the Great Basin types of the Sierra Madrean Flora to which it is also related.

As pointed out by Chaney (1925, 1938), the red-wood-forest occurred in the Miocene Epoch through not only the northern part of California but through the Pacific Northwest including the Columbia Basin, as well. With the subsequent uplift of the Cascade-Sierra Nevada Axis in late Miocene and early Pliocene, the rainfall and the temperature minimum in the Columbia Basin were reduced considerably, and mountain areas with new conditions were developed from Washington to California. It is possible that the present subalpine and montane members of the several species-complexes in *Ranunculus* are modified descendants of wider-spread populations once occurring on the forested Miocene plains. If this is the case, the species occurring today in the relatively little-modified coastal forest-belt and in the related area north and east of the Columbia Basin may be the prototypes of the species-complexes.

Four complex species or species-groups of *Ranunculus* are represented, each largely by a single type, in the relatively ancient and unmodified Northwestern Coniferous Forest. Each of these species (*R. occidentalis*, *R. Bongardii*, *R. orthorhynchus*, and *R. alismaefolius*) is replaced by varieties or closely-related species in the Cascade-Sierra Nevada Axis and the mountains in and about the northern Great Basin,⁷ as well as in southern Alaska, and in north-coastal California. Diffusion of the groups northward into the glaciated regions and southeastward into the Rocky Mountain System will be discussed directly below, but invasion southward into the California Oak Woodland will be taken up under that ecological type (cf. Sierra Madrean Flora below).

Reoccupation of the glaciated areas from Puget Sound northward to Alaska has been accomplished by the first three species-complexes listed above, all of which are in the section *Chrysanthae* of the subgenus *Euranunculus*, a group in which nearly all of the species are polymorphic, rich in biotypes, difficult of classification, and remarkably adaptable ecologically. The recent spread of the weedy European species, *R. acris*, *R. repens*, and *R. bulbosus* and their biotypes, into various parts of the

⁶ Lowlands of the Pacific Slope from Alaska to the red-wood-belt of northwestern California; inland north of the Cascade Mountains through British Columbia to the western side of Glacier National Park, Montana, and southward to parts of Idaho, where it reaches an elevation of 3,500 feet.

⁷ It is to be noted that in some cases these varieties have apparently followed some of the large rivers, which cut through the mountain-axis down to the coastal region, e.g. *R. Bongardii* var. *tenellus* along the Fraser, Columbia, and Klamath rivers and *R. orthorhynchus* var. *platyphylus* along the Klamath and Pitt-Sacramento rivers.

world, including North America, is illustrative of the aggressiveness of some species of the section *Chrysanthē*. The plastic *R. occidentalis-californicus-canus* complex is the Pacific Coast counterpart of these Old World species. The members of this species-complex occur in all parts of the Pacific Coast, and types ranging from subaquatic to distinctly terrestrial occupy such ecologically different areas as the cold Yukon Valley, the moderate and moist Northwestern Coniferous Forest, the warm and seasonally dry broad-leaf sclerophyll areas, and the hot and dry Pacific Prairie in the Great Valley of California. The spread of this group and of the *R. orthorhynchus* group and *R. Bongardii* northward into the glaciated areas in Washington, British Columbia, and southern Alaska and perhaps even beyond them into the Yukon Valley and the Aleutian Islands since the Wisconsin glaciation is in keeping with the characters of the section *Chrysanthē*. In marked contrast to the aggressive movement and rapid modification of this section is the lack of ability of the section *Epirotes* established in the arctic, alpine, and cordilleran areas to reoccupy the intervening glaciated areas (as discussed above) and the Pacific Subalpine and Montane Forests or the Northwestern coniferous forest.

Some of the groups mentioned above have spread southeastward into the Rocky Mountain System, where each is represented by satellites of the main bulk of the complex. Examples are as follows: *R. occidentalis* var. *montanensis* in southwestern Montana and adjacent Idaho and Wyoming and its possible derivative, *R. acriformis*, on the high plains of Wyoming and adjacent Colorado; *R. Bongardii* var. *tenellus* in the Bighorn Mountains in Wyoming and var. *Earlei*, an endemic in southern Colorado; *R. orthorhynchus* var. *platyphyllus* along the eastern side of the Great Basin as far south as the Wasatch and Uintah Mountains in Utah⁸; *R. alismaefolius* var. *montanus* in Southern Wyoming, Utah, and Colorado and *R. Collomae*, an endemic in southeastern Utah and adjacent Coconino County, Arizona.

3. *The Sierra Madrean Flora*.—According to Axelrod (1940, pp. 483–4), (1) the California Oak Woodland, (2) the California Chaparral, (3) the Southwestern Coniferous Woodland (juniper-pinyon type), (4) the Northern Desert (sagebrush type), and (5) the Southwestern Oak Woodland and Chaparral are derivatives of a Mexican woodland prototype, the Sierra Madrean Element, which migrated northward in the Oligocene Epoch and early Miocene and Middle Miocene times into the area now occupied by much of California and the Great Basin. With the uplift of the Sierra Nevada late in Miocene and early in Pliocene and the consequent

development of a continental climate east of the new mountain ranges, such characteristic California Oak Woodland species as the Digger pine (*Pinus Sabiniana*) disappeared from western Nevada. Subsequent development in California has produced a vegetation almost wholly distinct in species from the derivatives of the same prototype in the Great Basin,—that is, the Northern Desert and the Southwestern Coniferous Woodland. A sixth vegetation-type, the Pacific Grassland of the Great Valley of California is of obscure origin, and the original nature of the flora is not certain, although there is some evidence that it was a bunch-grass type. Nevertheless it is possible that in the main the Pacific Prairie should be classified floristically with the Sierra Madrean Flora. Only the California Oak Woodland and the Pacific prairie have significant *Ranunculus* populations of the dominant phase of the genus.

The California Oak Woodland supports a dense growth of annual grasses and herbaceous plants during the moist winter and spring growing season. Among the herbaceous perennials are *Ranunculi* of the section *Chrysanthē* and chiefly of the *R. occidentalis* complex. In keeping with the rest of the flora, all of the terrestrial *Ranunculi* are endemic to the woodland, or to it and part of an adjacent area. As may be expected in a relatively dry country, the number of species of *Ranunculus* is not as great as for the moist Northwestern Coniferous Forest, and the species present may be migrant derivatives of relatives in that area. It is to be noted that related *Ranunculi* are lacking in the other derivative vegetation-types of the Sierra Madrean Element, including the nearest modern equivalent, the Southwestern Oak Woodland and Chaparral of Arizona, New Mexico, and northern Mexico. Possibly this is due to the greater dryness of the present climate in these areas, but in any case a semi-xerophytic woodland of the probable original type is not a suitable place to look for much of the basic *Ranunculus* stock. It is more likely that the genus has spread into the warm and vernal moist California Oak Woodland chiefly from the adjacent Northwestern Coniferous Forest and that it has been unable to occupy the sagebrush, juniper-pinyon, and Southwestern Oak Woodland and Chaparral types, where winter rain stops abruptly at about the time when vernal temperatures finally rise high enough to favor growth.

A few species (of the *Ranunculus occidentalis* complex in the main) occur in the Pacific Prairie in the Great Valley of California. In all probability they are merely derivatives of relatives in the California Oak Woodland, which surrounds the Pacific Prairie.

4. *The Eastern Forest Flora*.—The Eastern Forest Flora includes the following subdivisions: the Deciduous Forests, the Southeastern River Bottom Forest, and the Southeastern Pine Forest. The best development of *Ranunculus* is in the Deciduous Forests.

⁸ Since the closely-related *R. macranthus* occurs in northern Arizona and southward to Mexico and Texas and since the *R. orthorhynchus-septentrionalis* complex is well-developed in Mexico, the direction of migration may have been northward perhaps in company with the Sierra Madrean Element in Oligocene and Miocene time. (Cf. the Sierra Madrean Flora. Cf. also discussion under Eastern Forest Flora).

The Eastern Forest Flora is characterized by several endemic species groups. The relationship of these compact groups to other types occurring in diverse directions is apparent, and the high percentage of endemic species indicates the antiquity of the present groups of *Ranunculi* in the Eastern Forests. The probable separate invasions of the prototypes of various complexes must have occurred long ago. The groups are as follows:

R. septentrionalis, *R. hispidus*, *R. carolinianus*, *R. fascicularis*, and their varieties, which are members of the group of species in the section *Chrysanth* characterized by long, straight achene-beaks. The species of this group are connected with their Pacific Northwestern relatives, the *R. orthorhynchus* group, by a chain of mutual relatives occurring across the southern part of the continent. *R. fascicularis* is related through its Texan variety *cuneiformis* to *R. macranthus*, which occurs in Mexico and Arizona as well as in Texas and which is barely distinguishable from some of the *R. orthorhynchus* group in the mountains of the Great Basin and on the Pacific Coast (cf. discussion under the Pacific Northwestern Flora above). *R. macranthus* has other relatives in Mexico, and the origin of the entire group of species now extending across the southern part of the continent may have been either on the Pacific Coast or in Mexico or possibly in the East (cf. footnote 8).

R. recurvatus (sect. *Chrysanth*) is related to and perhaps derived from *R. Bongardii* and its varieties, a group restricted to the Pacific Region and lacking such specialized features as the thickened, bulbous stem-base and the nectary-scale of the pocket-type (found in only *R. recurvatus* as far as the section *Chrysanth* is concerned (Benson, 1940).

R. abortivus, *R. micranthus*, *R. allegheniensis*, and *R. Harveyi* are an aberrant group in the section *Epirotes*, and they are the only plants of the section occurring in the forests of the East and Middle West. Since *R. abortivus* ranges from Alaska to British Columbia and into the Rocky Mountains as far south as Colorado as well as through the East and Middle West, it may be the prototype of the group. *R. arizonicus*, confined to Arizona and New Mexico, has been shown (Benson, 1940, p. 801) to be a connecting link between this group of species and *R. cardiophyllus*, which in turn shows relationship to still more orthodox species of the section. It is likely, therefore, that the four Eastern and Middle Western species are an offshoot in the section *Epirotes* derived from the Rocky Mountain Forest sub-center for the section.

R. ambigens is related to *R. Flammula*, a European species occurring as a relic in southeastern Newfoundland (discussed under part B above).

R. pusillus, *R. laxicaulis* (*R. oblongifolius* of authors), and their varieties. Any statement concerning the relationships of this group is deferred pending study of *Ranunculi* in other parts of the world.

Occurrence of *Ranunculus pusillus* in the northern California foothills might have been attributed

to casual introduction from the Southeast, had not Miss Carter's studies (Benson & Carter, 1939) shown conclusively that certain anomalous forms in the Sierra Nevada foothills are a distinct, related, endemic species, *R. alveolatus* Carter. This information strengthens the view that *R. pusillus* may be native and possibly relict in California. *R. alveolatus* may be another relic-type associated with it.

SUMMARY

The distribution of *Ranunculus* in North America north of Mexico is complicated by the existence within the genus of three phases, as follows: (A) widespread aquatic and palustrine types, which are not restricted in habitat by the same factors which limit terrestrial species; (B) relics of ancient floras; (C) the dominant phase of the genus, the species of which are largely in the subgenus *Euranunculus* and are nearly all terrestrial.

The relics of ancient floras include practically all the members of five of the seven native subgenera, and they may represent a phase of the genus with many highly differentiated types probably once well distributed through boreal regions and extending southward possibly to New Zealand and certainly throughout western North America. Other relics indicate relationship of the Pacific Coast Flora to that of Western Europe, and of the flora of Newfoundland and the Atlantic Coastal Plain to the Western European flora.

The species of the dominant phase of the genus may be segregated readily into geographic groups with distinct affinities to the following regional North American floras: the Circumboreal Flora, the Pacific Northwestern Flora, the Sierra Madrean Flora, and the Eastern Forest Flora. The genus is poorly represented or does not occur at all in the other floras, which are as follows: the Central Prairie Flora, the Mexican Desert Flora, and the Subtropical Flora.

The Circumboreal Flora includes the Arctic Tundra, the Alpine Tundra, and the Rocky Mountain Forests, which are bound together by the section *Epirotes*, which dominates all of them and which, in its typical form, is restricted to them. Absence of *Epirotes* in the Northern Coniferous Forest is explained by the lack of aggressiveness of the section or its apparent inability to fill in the areas denuded by glaciers in the Pleistocene Epoch.

The species-complexes of the Pacific Northwestern flora may have arisen along with differentiation of climates resulting from uplift of the Cascade-Sierra Nevada Axis in late Miocene and early Pliocene. The species occurring today in the relatively little-modified Northwestern Coniferous Forest may be the prototypes of the complexes, and apparently the derivative-members have migrated rapidly in all directions from the Pacific Northwestern center.

The Sierra Madrean Flora occupies most of California and the Great Basin and the lower mountains of Arizona, New Mexico, trans-Pecos Texas, and

TABLE 1. *Continued.*

	1. CIRCUMBOREAL FLORA	2. PAC. N.W. FLORA	3. SIERRA MADREAN FLORA	4. EASTERN FOREST FLORA	5. CENTRAL PRAIRIE FLORA
	a. Arctic Tundra	a. N.W. Conif. Forest	a. Calif. Oak. Woodl.	a. Deciduous Forest	a. Pine & Riv. For.
	b. Alpine Tundra	b. Sierra Forest	b. Pacific Prairie	b. S.E. Pine & Riv. For.	
	c. Rocky Mt. Forests	c. N.E. Conif. Forest	c. Northern Desert		
	d. N.E. Conif. Forest	d. S.W. Conif. Woodl.			
23. <i>R. Sardous</i>
C. Sect. <i>EPIROTES</i>
24. <i>R. abortivus</i> ^C
Var. <i>eucyclus</i> ^C
Var. <i>indivisus</i> ^C
25. <i>R. micranthus</i> ^C
26. <i>R. allegheniensis</i> ^C
27. <i>R. Harveyi</i> ^C
28. <i>R. arizonicus</i> ^C
29. <i>R. cardiophyllus</i> ^C
Var. <i>subsagittatus</i> ^C
Var. <i>coloradensis</i> ^C
30. <i>R. Eastwoodianus</i> ^C
31. <i>R. pedatifidus</i> ^C
32. <i>R. Sabini</i> ^C
33. <i>R. inamoneus</i> ^C
Var. <i>alpeophilus</i> ^C
Var. <i>subaffinis</i> ^C
34. <i>R. Alleni</i> ^C
35. <i>R. verecundus</i> ^C
36. <i>R. Eschscholtzii</i> ^C
Var. <i>Suksdorfii</i> ^C
Var. <i>eximius</i> ^C
Var. <i>trisetus</i> ^C
Var. <i>oxymotus</i> ^C
37. <i>R. adoneus</i> ^C
Var. <i>alpinus</i> ^C
38. <i>R. nivalis</i> ^C
39. <i>R. sulphureus</i> ^C
40. <i>R. Macauleyi</i> ^C
41. <i>R. Grayi</i> ^C
42. <i>R. pygmaeus</i> ^C
Var. <i>petiolatus</i> ^C
43. <i>R. rhomboidus</i> ^C
44. <i>R. glaberrimus</i> ^C
Var. <i>ellipticus</i> ^C
Var. <i>reconditus</i> ^C
45. <i>R. verticillatus</i> ^C
D. Sect. <i>FLAMMULA</i>
46. <i>R. Jovis</i> ^C
47. <i>R. Collomae</i> ^C
48. <i>R. alismae-folius</i> ^C
Var. <i>Hartwegii</i> ^C
Var. <i>alismellus</i> ^C
Var. <i>Lemmonii</i> ^C
Var. <i>montanus</i> ^C
49. <i>R. oresters</i> ^C
50. <i>R. ambigenus</i> ^C
51. <i>R. Flammula</i> ^B
Var. <i>ovalis</i> ^A
Var. <i>filiformis</i> ^A
Var. <i>samolifolius</i> ^C
52. <i>R. hydrocharoides</i> ^C
Var. <i>stolonifera</i> ^C
53. <i>R. Gormanii</i> ^C
54. <i>R. Populago</i> ^C
55. <i>R. lasiocaulis</i> ^C
Var. <i>mississippiensis</i> ^C
56. <i>R. pusillus</i> ^C
Var. <i>angustifolius</i> ^C
57. <i>R. alveolatus</i> ^C
E. Sect. <i>HECATORIA</i>
58. <i>R. hyperboreus</i> ^C
59. <i>R. natans</i> ^C
60. <i>R. sceleratus</i> ^A
Var. <i>multifidus</i> ^A

- * Introduced.
- † Sparingly introduced.
- ‡ Native, not endemic.
- § Native, not endemic, uncommon.
- § Endemic or nearly so in the subdivision.
- ¶ Endemic in the regional flora but not in the subdivision.
- Endemic in the regional flora, but rare in the subdivision.

*A, B, C.—A after the name of a species denotes a widespread aquatic, palustrine, or sub-palustrine type discussed in part A of the text; B a relic of an ancient flora discussed in part B, C a member of the dominant phase of the genus discussed in part C.

TABLE I. *Concluded.*

	1. CIRCUMBORAL FLORA				2. PAC. N.W. FLORA				3. SIERRA MADREAN FLORA				4. EASTERN FOREST FLORA				5. CENTRAL PRAIRIE FLORA			
	a. Arctic Tundra	b. Alpine Tundra	c. Rocky Mt. Forests	d. N.E. Conif. Forest	a. N.W. Conif. Forest	b. Sierran Forest	a. Calif. Oak. Woodl.	b. Pacific Prairie	c. Northern Desert	d. S.W. Conif. Woodl.	a. Deciduous Forest	b. S.E. Pine & Riv. For.								
Var. <i>longissimus</i> ^C
61. <i>R. Gmelinii</i> (Old World)
Var. <i>terrestris</i> ^A
Var. <i>prolificus</i> ^C
Var. <i>yukonensis</i> ^C
Var. <i>schizanthus</i> ^C
62. <i>R. flabellaris</i> ^A
II. Subg. CYRTORHYN- CHA																				
A. Sect. <i>HALODES</i>																				
63. <i>R. Cymbalaria</i> ^A
Var. <i>saximontanus</i> ^A
Var. <i>alpina</i> ^A
B. Sect. <i>EUCYRTO- RHYNCHA</i>																				
64. <i>R. ranunculi- nus</i> ^B
C. Sect. <i>ARCTERAN- THIS</i>																				
65. <i>R. Cooleyae</i> ^B
D. Sect. <i>PSEUDAPHANOSTEM- MA</i>																				
66. <i>R. hystericulus</i> ^B
III. Subg. CERATOCE- PHALUS																				
67. <i>R. testiculatus</i>	a	b	c	d	a	b	a	b	c	d	a	b	†							
IV. Subg. OXYGRAPHIS																				
68. <i>R. kamchaticus</i> ^B
V. Subg. CRYMODES																				
69. <i>R. glacialis</i> ^B
70. <i>R. Chamissonis</i> ^B
71. <i>R. juniperinus</i> ^B
72. <i>R. Andersonii</i> ^B
VI. Subg. BATRA- CHIUM																				
73. <i>R. hederaceus</i> ^B
74. <i>R. Lobbii</i> ^B
75. <i>R. aquatilis</i> (Old World)
Var. <i>capillaceus</i> ^A
Var. <i>eradicatus</i> ^C
Var. <i>hispidulus</i> ^C
Var. <i>calvescens</i> ^C
76. <i>R. circinatus</i> ^A
VII. Subg. PALLA- SIANTHA																				
77. <i>R. Pallasii</i> ^B
VIII. Subg. COPTIDIUM																				
78. <i>R. lapponicus</i> ^B
IX. Subg. FICARIA																				
79. <i>R. Ficaria</i>
TOTALS																				
SUBDIVISIONS OF RE- GIONAL FLORAS	a	b	c	d	a	b	a	b	c	d	a	b	5							
Introduced (*, †)	0	0	2	5	9	2	2	1	1	1	8	5	4							
Native, not endemic (‡, §, , °)	10	2	21	18	11	11	6	3	7	4	12	4	14							
Endemic or essen- tially so (§)	5	11	14	4	13	15	7	3	1	2	14	6	5							
Total native (‡, §, , °, §)	15	13	35	22	24	26	13	6	8	6	26	15	19							
REGIONAL FLORAS																				
Nos.	1		2				3				4		5							
Endemic or essential- ly so	34		28				13				20		5							
Total native	70		40				25				30		19							
Endemic species, domi- nant phase	30		25				10				20		5							
Significant species, dominant phase	40		33				17				21		6							

northwestern Mexico. The vegetation types differentiated since uplift of the Sierra Nevada are mostly poor in *Ranunculi*. However, the Oak Woodland and part of the Pacific Prairie in California have been invaded from the Northwestern Coniferous Forest. The warm, rainy winters have made invasion possible in this area, but it has not been possible in the other Sierra Madrean areas where winter rains are light and are over before the weather is warm enough for growth.

The Eastern Forest Flora is characterized by compact endemic groups of species with relationships to groups occurring in several directions. The high percentage of endemism indicates the antiquity of the probable separate invasions of the prototypes of these groups.

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THE ESTIMATION, ISOLATION, AND IDENTIFICATION OF AUXINS IN PLANT MATERIALS¹

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INVESTIGATIONS UPON the use of different types of extraction methods under varying conditions have indicated that the substances called auxins, which give a negative curvature of the *Avena* coleoptile, exist in plant tissues in more than one form. In the course of the present investigation, methods for the extraction of the less readily diffusible auxins have been studied. The auxin so extracted was isolated and identified.

The early experiments of Went (1928) proved that the substance influencing cell elongation in the *Avena* coleoptile could be separated from the plant by diffusion into agar. Later investigators proposed other methods of effecting extraction, which may be classified according to general type as follows:

- (1) Solvent extraction (summary by Avery [1939]; Avery, *et al.* [1940]).
- (2) *In vitro* chemical or enzymatic treatment followed by solvent extraction.
 - (a) Alkaline hydrolysis (Kögl, *et al.*, 1934a and b; Avery, *et al.*, 1941; Haagen-Smit, *et al.*, 1941).
 - (b) Lipase hydrolysis (Kögl, *et al.*, 1933b).
 - (c) Chymotrypsin (Skoog and Thimann, 1940).
- (3) Biological digestion (Kögl, *et al.*, 1933b).

Application of the extraction methods of the various investigators has indicated that the auxin present in plant materials exists in at least two forms: a readily diffusible or "free" form and a less readily extractable or "bound" form. Recently, Thimann and Skoog (1940) have made further distinctions between different types of extraction behavior in the materials which they studied (*Lemna*, *Nicotiana* callus, *Phaseolus* nodules, *Avena*). Their tentative classification of the forms in which they consider the auxin to be present is as follows:

- (1) *Free*, readily diffusible.
- (2) *Naturally bound*, apparently an auxin-protein complex.
- (3) *Reversibly fixed*, becomes non-extractable upon drying and is extractable upon rewetting.

Different plant materials may behave quite differently as regards the extraction of auxin, and it has

been impracticable heretofore to formulate a simple, inclusive, quantitative extraction method. For example, Thimann and Skoog found that repeated solvent extraction upon *Lemna* continued to remove auxin but that this was not the case with *Avena* coleoptiles. They concluded, as did van Overbeek (1941), that solvent extraction recovers, in the case of *Avena*, only a fraction of the total auxin which the coleoptile tips would have yielded had they remained alive. Skoog clearly showed in *Avena* seedlings the existence of an inactive substance originating in the seed and activated at the coleoptile tip. Van Overbeek (1941) studied the relative amounts of auxin obtainable from *Zea* and from *Avena* coleoptile tips by exhaustive diffusion as compared to those removed by exhaustive solvent extraction. He found that "potential" auxin, as measured by exhaustive diffusion, is considerably greater than that obtainable by complete extraction. The "potential" auxin he ascribes to continued formation from precursor. Further, Went (1942) has studied the movement of precursor to the tip ends of decapitated *Avena* coleoptiles and has found an increasing accumulation of precursor. The combined evidence of these investigators indicates that at least one precursor is something other than a non-motile auxin-protein complex.

Still another factor in the problem of the forms of auxin in plant materials has been introduced by the recent work on inhibitor substances. Evidence for a definite inhibitor substance was first presented by Stewart, Bergren, and Redemann (1939). Further experiments carried out by Redemann showed that an alkaline hydrolysis of the purified inhibitor substance, obtained from young radish plants, produces a substance effecting negative curvatures in the *Avena* test, rather than the positive curvature characteristic of the original inhibitor. Evidence was obtained indicating that the growth substance so formed was indole-3-acetic acid; consequently, the radish inhibitor may be considered as a "potential" auxin source. Goodwin (1939) and Larsen (1939) have also published data indicating the existence of growth inhibitors in plant extracts.

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In addition to the problem of the form of auxin in plant materials, the question of the identity of the active substance or substances is of interest and importance. Certain identification must be based upon actual isolation in pure condition of the physiologically active substances. However, a number of differentiation tests have been devised and used, which, while not serving as means of definite identification, have had important bearing upon phytohormone studies. A frequently used differentiation test has been that of Kögl, Haagen-Smit, and Erxleben (1934a), which depends upon the differing stabilities of auxin-a, auxin-b, and indole-3-acetic acid towards boiling with acid and boiling with base. In most cases, however, the applications of this method have been to such relatively impure extracts that uncertainty regarding the validity of the results has been inevitable. Another differentiation method is the diffusion-constant method used by Went (1928).

The present investigation was undertaken with two purposes in mind: first, to devise a satisfactory inclusive auxin extraction method, and second, to identify the physiologically active substances so extracted. Such an extraction method is one which would extract, by an *in vitro* method, auxin in all its forms, regardless of the particular entities concerned. Further, it is desirable that the extract so prepared be in a form suitable for identification of the active substances by isolation.

The extraction process finally adopted grew out of work involving the biological digestion method of Kögl, Haagen-Smit, and Erxleben (1933b). This method, although of necessity limited in application, is capable of giving the highest hormone content of a plant material as compared to other methods. In this procedure, the human organism is used to extract the growth substance, the extracted auxin being found in the urine. The method had its origin in the observation that the urine showed a peak in auxin activity about two hours after each meal and that during days of fasting the urinary auxin activity remained constant. Experiments have indicated that the rise in the auxin activity of the urine could be related directly to the ingestion of foodstuffs, i.e., to auxin arising from the ingested material.

In the present studies, biological digestion of wheat was found to give a yield of auxin higher than that obtained by other extraction methods. This yield was then used as a criterion of the highest amount of growth hormone available from wheat. A systematic study of *in vitro* methods for obtaining auxin yields comparable to those obtained by biological digestion was then carried out. Having arrived at such an *in vitro* procedure, the method was applied to other materials, and isolation and identification studies of the active material were undertaken.

PROCEDURE.—Assay method.—All auxin determinations were made by the standard *Avena* test (Went and Thimann, 1937, pp. 27–42), using an interval of three hours between decapitations and a period of ninety minutes between blocking and photographing. The minimum number of *Avena* test plants used

for any single determination was twelve, although in many instances twenty-four or thirty-six plants were used.

In every case the data presented are the results of a single test. Each test, however, was preceded by a number of preliminary experiments to establish the maximum and minimum range for each substance.

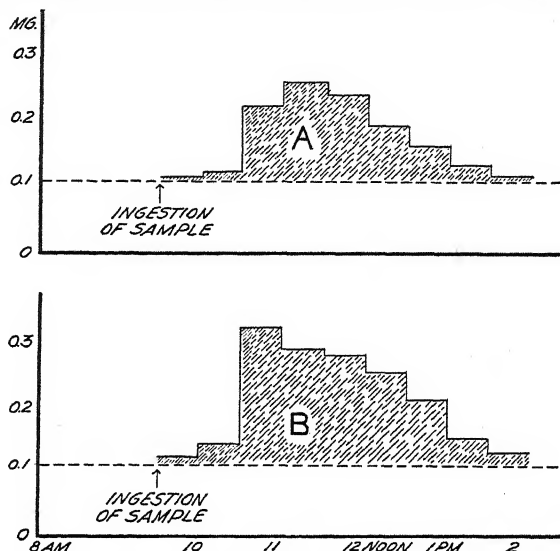


Fig. 1. Half-hour excretion of auxin in terms of indole-3-acetic acid. The excretion of auxin is determined every half hour after the ingestion of the sample. The shaded area represents the total amount of excreted auxin due to the sample, A, from medium hard wheat yielding 6.5 mg. per kilogram of wheat, B, from soft wheat yielding 9.8 mg. per kilogram of wheat. The dotted line represents the fasting level in each case.

Biological digestion method.—In agreement with the observations of earlier investigations, it was found that the increase in urinary auxin activity was several times more than could be accounted for by assay of the foodstuff. The desirability of duplicating this effect *in vitro* was evident, and consequently experiments towards this end were undertaken.

As a first step in this direction, the maximal amounts of auxin obtainable from wheat by the biological digestion method were determined. A typical experiment is outlined as follows:

1. Urine accumulated overnight passed and discarded at 5:30 a.m.
2. 100 cc. water ingested at 6:00 a.m. and a like amount each half hour thereafter until 1 p.m.
3. First urine sample collected at 7:30 a.m., and subsequent samples collected at half-hour intervals until 1:30 p.m.
4. 100 grams of soft white wheat kernels ingested at 9:00 a.m.
5. The volume of the urine at each collection was measured, and the sample was then diluted sufficiently to give a curvature of 5 to 15 degrees in the standard *Avena* test.

Assay of the separate urine samples collected showed that the peak of the yield of auxin was ob-

TABLE 1. Auxin yields from "medium hard" wheat upon treatment with enzymes.

Enzyme ^b	pH-Maintained	mgm./ml./Enzyme used	Auxin yield ^a mgm./kgm. wheat
Pepsin	1.5-2.5	12.5	0.16
Pepsin	1.5-2.5	25.0	0.20
Urease	6-7	2.0	0.40
Lipase (gastric)	5-6	12.5	0.22
Lipase (gastric)	5-6	25.0	0.18
Trypsin	7-8	0.5	0.35
Trypsin	7-8	1.0	0.22
Chymotrypsin	8.0	0.5	0.67
Chymotrypsin	8.0	1.0	0.76
Biological digestion	6.9
Water extraction	0.2

^a In terms of indole-3-acetic acid, calculated according to van Overbeek (1938b).

^b Buffered to optimum pH for each enzyme, with phosphate-acid phosphate buffer and held at 35°C. for thirty hours.

tained within two to two and a half hours after ingestion of the wheat (fig. 1). The total auxin yield as obtained by this method was found to be 9.8 mgm. of active material, calculated as indole-3-acetic acid, per kilogram of ingested wheat. This figure has been corrected for the average total yield obtained in the urine in corresponding experiments in which wheat was not ingested.

Since the ingestion of tryptophane in the course of similarly performed experiments showed no auxin increase above the average level for a fasting day, oxidative deamination of tryptophane proceeds too slowly to contribute appreciably to the above results. For this reason it is believed that the above value of 9.8 mg. per kgm. represents free, bound and/or precursor auxin in the whole wheat ingested. The value 9.8 mgm./kg. was obtained for "soft" wheat only. A large variation was found in the maximum yields obtainable from different wheats, "medium hard" wheat giving a maximum yield of 6.5 mgm./kgm. (fig. 1). (The terms "soft" and "hard" are used in reference to low and high protein content of the wheat.) It is of interest to note that a solvent extraction method (water, ether) will yield 0.5 to 1.0 mgm. of auxin per kgm. of soft wheat, and a scarcely detectable amount from "hard" wheat.

This method served as a criterion for the highest auxin yield from whole wheat. *In vitro* studies upon this material were then undertaken.

Extraction and enzyme hydrolysis.—Three methods of attack were used: (1) search for suitable extractants; (2) use of enzymes; and (3) treatment with acids and bases. The efficiency of each method employed was judged by the quantity of auxin obtained in relation to that obtained by the biological digestion method.

Butyl alcohol saturated with water served relatively well for extraction, though not as well as either the purified ether method of van Overbeek (1938a) or the absolute alcohol method of Avery (1939). Since a large number of other extractants tried

proved even less successful, an enzymatic method of liberation of auxin was then tried.

Several enzymes were investigated, including some of those commonly found in the digestive tract; namely, ptyalin, pepsin, chymotrypsin, lipase, amylase, urease, papain, tyrosinase, taka diastase, and, in one case, the mucosa pylorus of the rabbit, a source of mixed enzymes. Each wheat sample, together with the enzyme under trial, was incubated for twenty-four hours at the optimum pH of the enzyme in question at a temperature of 35°C. The sample was then centrifuged, and the clear supernatant liquid was tested for its auxin content.

Of the enzymes tried, only chymotrypsin, a gift to this laboratory from Dr. Northrop, gave a yield representing any significant increase over a blank determination. This increase is probably associated with hydrolysis due to the alkaline condition of the medium, as is indicated in the following examination of the pH effect. Table 1 shows the effects of the various enzymatic treatments.

Alkaline hydrolysis.—Kögl, Haagen-Smit, and Erxleben (1934b) found that sodium ethylate smoothly hydrolyzed the bound hormone in corn oil. Hydrolysis by this scheme was carefully investi-

TABLE 2. Effect of pH on auxin yield. (Room temperature, 48 hours.)

pH	Auxin ^a mg./kgm. wheat
1.5	0.26
2.8	0.12
3.8	0.09
5.3	0.38
6.3	0.46
7.1	1.25
8.6	3.60
9.4	3.80
10.5	6.96
12.0	1.00
13.0	0.00

^a Computed as indole-3-acetic acid.

gated to ascertain if whole wheat might respond in a similar manner, but without success. However, an extension of this method did prove to be suitable, namely, an alkaline aqueous hydrolysis, as described by Avery and co-workers (1941). Haagen-Smit, Leech, and Bergren (1941) showed a similar increase in extractable auxin by use of an alkaline hydrolysis.

Table 2 shows a typical experiment upon the effect of pH on the quantity of auxin liberated from a sample of medium soft wheat, as measured by the standard *Avena* technique.

The optimal pH having been found to be at 10.5, the effect of other variables was studied.

In table 3 is shown a typical experiment upon the effect of time on the quantity of hormone liberated at pH 10.5, as determined by the standard *Avena* test.

TABLE 3. *Effect of time upon auxin yield. (Room temperature, pH 10.5.)*

Time in hours	Auxin ^a mg./kgm. wheat
0.5	0.0
1.0	0.06
2.0	0.14
12.0	0.20
16.0	0.22
20.0	0.26
40.0	3.70
48.0	6.96
60.0	6.00
90.0	4.40

^a Computed as indole-3-acetic acid.

As the effect of increased temperature (table 4) was of small magnitude, room temperature was adopted for routine study of the alkaline hydrolysis effects. Above 60°C., the auxin yield was considerably less, especially over the long periods of hydrolysis employed.

TABLE 4. *Effect of temperature upon auxin yield. (pH 10.5, 36 hours.)*

Temp. °C.	Time hrs.	Auxin ^a mg./kgm. wheat
20	36	3.4
35	36	4.1
45	36	4.3
60	36	4.2
75	36	3.0

^a Computed in terms of indole-3-acetic acid.

After considering the results obtained by treating dormant tissues such as wheat, corn, and oat kernels, under various conditions, the following procedure was selected as a method giving results comparable to the yields of the biological digestion method.

Method.—Twenty-five grams of dry whole wheat are placed in a 125 ml. heavy walled Pyrex glass

centrifuge bottle and soaked for four hours with 50 ml. of NaOH solution adjusted to pH 10.5. At the end of this period, the contents are emptied into a mortar together with half the weight of specially cleaned sand (washed in steam, alkali, acid, and water, then dried). They are thoroughly ground and then carefully transferred to the original centrifuge bottle, the residue remaining with the mortar and pestle being washed into the tube to make a final volume of 100 ml. The pH is readjusted to 10.5 by dropwise addition of a concentrated solution of NaOH. One ml. of toluene is added, and the bottle is stoppered with a cotton plug and is set aside out of direct light at room temperature (about 20°C.) for a period of 48 hours. The pH is readjusted to 10.5 ten hours after starting the experiment. This operation is repeated at four-hour intervals during the hydrolysis. At the end of forty-eight hours, the mixture is adjusted to pH 6.5 and centrifuged. The clear supernatant liquid is appropriately diluted for assay by the *Avena* test.

ACID-BASE SENSITIVITY OF THE ALKALINE HYDROLYSATE.—Although it was understood that the acid-base sensitivity test, as outlined by Kögl, Haagen-Smit, and Erxleben (1934a), would not serve to determine with certainty the identity of the auxin liberated upon alkaline hydrolysis, experiments were made in this direction to obtain preliminary information.

A typical experiment may be summarized as follows:

- Two 20-gram portions of yellow cornmeal were mixed with 200 ml. of water. One of these mixtures was held as a control; the other was adjusted to pH 10.5 and held there for the hydrolysis period of forty-eight hours.
- At the conclusion of this period, the alkaline hydrolysate was divided into three portions, treated as follows:
 - One portion was held as a control.
 - One portion was mixed with an equal portion of 10 per cent sulfuric acid and boiled under reflux for three hours. The solution was then neutralized for *Avena* testing.
 - The remaining portion was mixed with an equal portion of 10 per cent potassium hydroxide and boiled under reflux for three hours.
- The following results were obtained upon assay for auxin by the *Avena* procedure (the figures represent auxin yield in terms of mgm. indole-3-acetic acid per kgm. of cornmeal):
 - Cornmeal control..... 1.8 mgm.
 - Cornmeal hydrolysate control..... 13 mgm.
 - Acid treated cornmeal hydrolysate..... 0 mgm.
 - Base treated cornmeal hydrolysate..... 10 mgm.

It is to be seen that the auxin activity is lost upon acid boiling (within practicable testing dilution limits) and is substantially retained with the treatment by base. This behavior is indicative of the presence of indole-3-acetic acid in the hydrolysate. These results are consistent with the findings of Avery, *et al.*, 1941.

Despite the results of these experiments, it was considered desirable to conduct the isolation work

toward the isolation of auxin-a and -b, with indole-3-acetic acid as an auxiliary aim, rather than toward an isolation directed solely at the recovery of indole-3-acetic acid.

ISOLATION WORK.—Yellow cornmeal, fresh from milling was used as a convenient source material for isolation and identification studies, after preliminary experiments had been carried out in the manner previously described for wheat. A typical experiment may be summarized as follows: standing at pH 10.5 for forty-five hours gave 13.0 mg./kgm. (calculated as indole-3-acetic acid) as against 1.8 mgm./kgm. in a control sample standing with water at pH 7.0.

For isolation purposes, the obvious method for liberation of auxin from the cornmeal would be essentially the application of hydrolysis with a dilute aqueous alkaline solution as described in the above testing procedure. However, it proved to be more convenient for large scale extractions to carry out the hydrolysis at pH 10.5 in an alkaline 50 per cent acetone-water (acetone purified by distillation over alkaline KMnO_4). The pH was adjusted to 10.5 with 6N NaOH, and every six hours during the hydrolysis period the mass was stirred and the pH readjusted to 10.5. After fifty hours the mass was pressed, washed, and the collected filtrates were filtered through a layer of sodium chloride. This filtration had as its primary purpose the separation of the filtrate into an acetone phase and a water phase. It had the further advantage of breaking down fat-water emulsions, as well as of clearing the mixture of extraneous materials.

The aqueous portion was washed five times with acetone, the volume of acetone in each washing being one-fourth that of the water. After combining the first acetone phase with the acetone washings, the bulk of the acetone was distilled off at atmospheric pressure, and the residue was evaporated under vacuum to a volume suitable for ether extraction. The washed water layer was discarded, after being found by assay to be free of auxin.

Two large scale isolations were carried through this procedure, the first (lot number one) being upon 270 kilograms of cornmeal and the second upon 45 kilograms. Lot number 1, starting with the acetone soluble residue, was carried through the original isolation plan of Kögl, Haagen-Smit, and Erxleben (1933a) for the isolation of auxin a and b from urine concentrates.

In the course of the isolation from lot number 1, the ligroin (90° – 120°) extraction was made more thorough than has been customary in previous work (in this case twenty-one refluxings of twenty minutes, each treatment of the 10-gram residue being with 500 ml. ligroin). The soluble fraction weighed 1.6 grams and contained 102 mgm. of active substance calculated as indole-3-acetic acid; the insoluble weighed 5.5 grams and contained 160 mgm. of active substance, computed as indole-3-acetic acid. Upon lactonization (heating with a 1.5 per cent solution of hydrogen chloride in anhydrous methyl

alcohol), the bulk of the activity of the ligroin insoluble fraction was lost, and from the lactonized ligroin soluble fraction pseudo-auxin-a finally was isolated following the procedure of Kögl, Haagen-Smit, and Erxleben (1933a). As has been reported (Haagen-Smit, *et al.*, 1941) this crystalline material melted at 196 – 7°C ., with sintering beginning at 173°C . Kögl, Koningsberger and Erxleben (1936) give the melting point of pseudo-auxin-a as 193 – 4°C . with sintering at 176°C . This points to the presence of some auxin-a in the original starting material, which rearranged to pseudo-auxin during the isolation procedures.

In view of these results, it was decided to modify the procedure to include an even more exhaustive ligroin extraction as early in the procedure as possible. Accordingly, the second isolation was undertaken. The procedure is summarized in the following table:

SCHEMATIC DIAGRAM OF SECOND CORNMEAL ISOLATION.—(The first figure in the bracket following the fraction is the weight of the fraction and the second is the amount of auxin, calculated as indole-3-acetic acid.)

Yellow cornmeal	45 kgm.	(900 mg.)
↓ Alkaline hydrolysis	50 per cent acetone-water	
Extract		
▼ Salting out—acetone washing of aq. layer		
Residue (Acetone soluble)		
▼ Extraction with ether		
Residue (Ether soluble)	123 gm.	(200 mg.)
↓ Fractionation with bicarbonate solution and extraction of acid fraction with Pet. Ether (30 – 60°C .)		
Insol. fraction	15 gm.	
▼ Fractionation with bicarbonate		
Bicarbonate soluble (acid fraction)	12 gm.	(200 mg.)
↓ Absorption purification by precipitation of $\text{Ca}(\text{OH})_2$ in the solution		
Filtrate fraction	7.8 gm.	(180 mg.)
▼ Fractionation with benzene		
Main residue	4.5 gm.	
▼ Extraction with Pet. Ether		
Insoluble fraction	4.0 gm.	
↓ Exhaustive extraction with Ligroin (90 – 120°C .) (Twenty 30-minute refluxings with 800 cc. Ligroin)		
Soluble fraction	1.7 gm.	50 mg.
↓ (Insoluble fraction only very slightly active—discarded). Lactonization (1.5 per cent solution of hydrogen chloride in methyl alcohol)		
Ester-lactone fraction	1.2 gm.	26 mg.
↓ Distillation in high vacuum		
Fractionation by temperature control at constant pressure (0.01 mm.)		
Principal fraction (distilling up to bath temperature of 125°C .)	472 mg.	11 mg.
↓ Fractionation with bicarbonate in aqueous acetone		
Neutral fraction	326 mg.	10 mg.
▼ Hydrolysis at room temperature with 3N KOH		
Acid fraction	250 mg.	7 mg.
▼ Extraction with Xylene		

Soluble fraction 122 mg. 7 mg.
 ↓ Crystallization began after standing 3 months at 2°C.
 ↓ Recrystallization after standing twelve months at 2°C.
 Crystalline, colorless plates MP. 164–5°C. No melting point depression on mixing with synthetic indole-3-acetic acid. (MP. 164–5°C.) Crystals identified as indole-3-acetic acid.

DISCUSSION.—By isolation and identification three auxins have been shown to be present in higher plants, *nl.* auxin-a and -b and indole-3-acetic acid. Auxin-a and -b were isolated by Kögl, *et al.* (1934b), from corn oil and from sprouted barley (malt). Usually these materials contain very little auxin, which can be extracted by treatment with water. After trial of many samples Kögl and co-workers were fortunate in obtaining several hundred kgs. of malt and barley with exceptionally high auxin content from which auxin-a and -b were isolated. Attempts to produce artificially malt with such a high auxin content failed, although it was observed that the amount of water-extractable auxin increased with the time of germination. We have tried here to obtain oils or seeds with similar high free auxin content, but did not succeed. Attention was then focused upon the diet experiments carried out by Kögl, *et al.* (1933b), whereby the auxin excreted was much more than could be accounted for on the basis of ingested food materials. Our diet experiments showed that whole wheat produced this increased yield on biological digestion, whereas with solvent extraction only a fraction of the amount excreted in the urine could be determined. Studies upon the *in vitro* duplication of the effect led to a procedure of alkaline hydrolysis giving the same yields of auxin as the biological digestion experiments. An extension of this process using aqueous alkaline acetone as hydrolyzing agent furnished a starting material for isolation work. Although a considerable increase in total auxins results from the hydrolysis, the material is still not comparable to the auxin-rich corn oil and barley available to Kögl and co-workers. This hydrolyzed material contains many more impurities than the extracts containing the free auxins. Losses were also due to a tendency of the solution to form emulsions. This behavior increased the time necessary for the extractions considerably, thereby reducing the amounts of auxin-a and -b, since both substances are known to rearrange readily with the formation of the pseudo-auxins. Another important factor in these losses is the hydrolysis under alkaline conditions. Treatment with alkali decomposes both auxin-a and -b, whereas indole-3-acetic acid is stable under these circumstances. Therefore, it is not surprising that auxin-a could not be isolated from this hydrolysate; we did, however, obtain a small amount of the inactive pseudo-auxin-a showing the presence of auxin-a in the original material. The major portion of the auxin activity in hydrolyzed corn appears to be due to indole-3-acetic acid as concluded from stability tests to alkali and acid. This is in accordance with the work of Avery, *et al.* (1941). The

presence of indole-3-acetic acid was then definitely established by isolation and characterization by melting point and mixed melting point with synthetic material. From the work of Kögl, *et al.*, we have to conclude that in corn with exceptionally high free auxin content the water-extractable auxins contain a considerable amount of auxin-a and -b. The free and bound auxins in corn consist largely of indole-3-acetic acid and some auxin-a.

When we want to determine the presence and type of the free and bound auxins, isolation followed by characterization is in most cases not possible through lack of material or low auxin content. In such cases we have to satisfy ourselves with less specific methods, such as molecular weight determination by diffusion and acid and base sensitivity tests. In this respect the difference in physiological effect of auxin-a and -b and indole-3-acetic acid might be of importance. Experiments by van Overbeek (1936) with crude auxin-a preparations, which were later confirmed and extended by Koningsberger and Verkaarik (1938) with pure auxin-a, showed that decapitated oat seedlings responded with decreased growth to light if auxin-a is applied and not when the plants elongate under the influence of indole-3-acetic acid. This behavior found an explanation in the different chemical structure of auxin-a lactone and indole-3-acetic acid. Auxin-a lactone, which is in water solution in equilibrium with auxin-a, is, under influence of light, changed into the physiologically inactive lumi-auxin-a lactone (Oppenoorth, 1939). Such a change is not possible in the molecule of indole-3-acetic acid.

SUMMARY

A method of auxin extraction comparable in completeness of extraction to the *in vivo* biological digestion method is described as being a hydrolysis at pH 10.5 for forty-eight hours at room temperature.

Isolation of the auxin extracted from cornmeal by this method yielded crystals melting at 164–165°C., identified as indole-3-acetic acid.

Pseudo-auxin-a was isolated from the hydrolyzed cornmeal, which shows the presence of auxin-a in the original material.

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THE PRODUCTION OF SPORIDIA OF *CRONARTIUM RIBICOLA* ON CULTIVATED RED CURRANTS IN RELATION TO INFECTION OF WHITE PINE¹

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IN a previous paper (Snell, 1941b) the results of studies of white pine near cultivated red currants were presented, showing that such pines became infected very rarely by sporidia of *Cronartium ribicola* and only at short distances, unless there were also wild *Ribes* within infecting distance. The present paper deals entirely with the blister-rust pathology of garden currants as compared with some other species of *Ribes*, its relation to the control of potential sporidium production and the determination of distances at which pines can be infected from these species.

For at least twenty years, it has been recognized that cultivated black currants are much more dangerous to white pine² and at much greater distances than are other eastern species of *Ribes*, but there has been no thorough-going study of the reasons for this. The only direct answer that is more than a suggestion or a vague generality has been hidden in a little-circulated and poorly known publication (Spaulding, 1920, p. 11), and although blister-rust workers have tacitly assumed its application to the question (*cf.* York, Snell and Rathbun-Gravatt, 1927, p. 509) no

attempt has ever been made to apply this answer to other species of *Ribes*, let alone the red currants³ and the important red-currant problem.

One answer to this question has been that the greater susceptibility of *R. nigrum* results in the formation of teliospores and sporidia of greater vigor than is possible upon other species of *Ribes* which are less congenial to the rust. Spaulding (1922b, p. 224) stated that the telia on *R. nigrum* are "of maximum vigor in germination and production of sporidia." From further work (Spaulding, 1925, and Spaulding and Rathbun-Gravatt, 1925 and 1926), there seems to be little doubt that this is true, and it is quite likely that the same is true of the vigor of germination and ability of the sporidia to infect (York, Snell and Rathbun-Gravatt, 1927, p. 508, and Lindgren and Chapman, 1933), but the evidence is neither abundant nor clear, nor as to the latter point, proof against justifiable criticism. Unless the differences in virility of the teliospores and sporidia should be very much more pronounced than the evidence indicates, it is difficult to understand why the

³ In this paper, as in those papers preceding this one (Snell, 1941a, footnote 1, and Snell, 1941b), "red currants" refers to the garden red currants of hybrid origin customarily referred to *Ribes sativum* (Rchb.) Syme. Similarly, by "black currants" is meant the European black currants, *R. nigrum* L. The swamp red currant, *R. triste* Pall., and the wild black currants, *R. americanum* Mill., are not treated in this paper.

¹ Received for publication February 28, 1942.

² The phrases "danger to white pine," "dangerous distances," "*Ribes* causing infection of white pine," are used to express as concisely as possible the infection, or possibility of infection, of white pine by sporidia of the blister-rust fungus and the distances to which these sporidia can be carried to infect pines.

sporidia from *R. nigrum* will infect large percentages of pine at 5,000 to 6,000 feet and those from the wild gooseberries few or none beyond 800 to 900 feet under similar circumstances, especially since the evidence from most of the experiments so far performed⁴ indicates that the sporidia from any species of *Ribes* can withstand exposure to drying and sunlight for periods long enough to cover their passage over several to many miles through the air (Spaulding, 1925, and Spaulding and Rathbun-Gravatt, 1926).

Another explanation as to why cultivated black currants are dangerous at greater distances than other species of *Ribes* is based upon the tremendously larger volume of sporidia produced at a given location on *R. nigrum*, in consequence of which some viable sporidia will be carried to a distance greater than is possible from the smaller volumes of sporidia produced on other species of *Ribes*. In other words, this idea suggests the possibility of a more or less direct relation between numbers of sporidia produced at a given source and the distance to which the sporidia will be carried by the wind. In fact, Pennington (Spaulding, 1920) early formulated this explanation as follows: "... under given conditions the number of infections in pine varies directly with the amount of *Ribes* leaf surface and inversely as the square of the distance from *Ribes*." Obviously, "amount of *Ribes* leaf surface" is meaningless, but implies the number of sporidia produced on the infected leaf surface. Pennington's statement, therefore, means that the distance of dissemination of the sporidia varies directly with the square root of the number of sporidia produced on a given species of *Ribes*.

Whether or not this statement of the relationship is valid, whether the relationship is one of square roots or is a linear one, will not be argued here. There is at least another possibility that must be taken into consideration, if it is not the most important consideration. It is, in a way, a corollary of the preceding mathematical explanation, and emphasizes more than mere numbers of sporidia produced, the volumes of *viable sporidia necessary for infection*. It may be, and very likely is, true that there is a "threshold" or "quantum" relationship between sporidium production and infection. It is certain that huge numbers of sporidia are produced for every infection of pine. It is possible that wild gooseberries do not produce enough sporidia to provide the proper quantity for more than sporadic infection at distances over 900 feet. It is possible that in the case of

⁴ In some very early experiments, York was unable to obtain germination of sporidia caught 2,400 feet from black currants, and he also found that sporidia from this same source remained viable less than ten minutes at a relative humidity of 90 per cent between 22°C. and 25°C. (Spaulding, 1920, p. 11). Spaulding and Rathbun-Gravatt, however, later showed that in no tests did sporidia from any host fail to germinate after one-half hour at any saturation deficit, and in most tests, sporidia from all hosts germinated after drying for periods of nine to twenty-six hours (Spaulding and Rathbun-Gravatt, 1926).

In connection with the general problem, McCubbin's work (1918) on the rate of fall of spores should be kept in mind.

red currants this threshold number or quantum is not even reached.

No work has been done by the writer on the vigor or germinability of the sporidia, but a study has been made of volumes of sporidia produced by the different species of *Ribes*. This has involved a detailed study of the number of leaves to the bush and to the plot, the total leaf area of bushes and groups of bushes, the number of telia and hence of sporidia produced, in other words, the volume of infective material endangering white pine, or what has been called the "hitting power" of different species of *Ribes*.

BUSH SIZE AND LEAF AREA.—Red currants.—Leaf counts have been made of about seventy-five patches of red-currant bushes. Bushes in very poor condition may have as few as 200 leaves. A few bushes have been found with as many as 22,000 leaves. Many patches will have bushes with 10,000 to 15,000 leaves each, while the average garden bush was found to bear 3,000 to 10,000 leaves. Two large canes on a bush in Port Henry were found to bear 1069 and 1,137 leaves respectively.

The areas of individual leaves vary from a fraction of a square inch up to 14.3 square inches.⁵ The average of many measurements was 3.346. Some bushes will have a large number of average or small-sized leaves, and others a smaller number of very large leaves. Any bush will have large leaves on the new canes and smaller leaves on the older canes.

Black currants.—The number of leaves borne by black-currant bushes is about the same as for red currants, up to 20,000 or perhaps more on very large, vigorous bushes, with the average bushes bearing 4,000 to 10,000 leaves. The areas of individual leaves measured in recent years were found to vary from 1.5 to 14.3 square inches, with an average of 4.136. Spaulding (1922b) stated that this species produces an area of leaf surface that is maximum for the genus (that is, maximum for eastern species).

The leaf areas of all the bushes available from 1936 to 1938 ranged from 2,606 to 33,770 square inches, with the average bushes having areas of 16,000 to 25,000 square inches.

Wild gooseberries.—Bushes of *R. cynosbati* L. counted from 1935 to 1938 ranged from 15 to 20 leaves up to 18,000. Good-sized bushes averaged 7,000 to 9,000 leaves, while very large ones might have 50,000 or more leaves.

The areas of individual leaves vary from 0.22 to 6.83 square inches, although leaves over 2 square inches in area are rare except on new shoots growing in the shade. In the open, the leaves will average, on different bushes, from 0.45 to 0.75 square inches; in the shade, as much as 1.4 square inches.

Good-sized bushes have been found to have a total leaf area of 5,000 to 6,000 square inches, with the largest probably having 12,000 to 25,000 square inches.

⁵ The areas of large numbers of leaves were obtained from curves of area against width, prepared for four species of *Ribes* after the manner of Caruthers (1929).

In a half acre of one pasture that could be taken as a typical unit because of the distribution of the bushes as well as their concentration, there were 28 bushes with a total leafage of 93,000, an average of 3,320 leaves per bush. In 1938, the largest leaves in this pasture were 3.5 square inches, but few were more than 1.4 square inches in area, and the average of several bushes was 0.73 sq. in. The total leaf area for the half acre of pasture was 67,890 square inches. In 1940, leaves from these same bushes averaged only 0.455 sq. in. in area.

Bushes of *R. rotundifolium* Michx. usually have more leaves of smaller size (in a rough ratio of 11 to 6 or 8) and a smaller total leaf area per bush than is the case with *R. cynosbati*. Quite ordinary bushes have 15,000 to 22,000 leaves each, while large bushes 6 feet high or over may have 50,000 leaves.

The largest leaves found in 1938 had an area of 1.6 square inches, in 1940, 2.66 square inches. In 1938, the average of several hundred leaves was 0.446 sq. in.; in 1940, 0.415 sq. in. The total area of ordinary bushes ran from 7,000 to 11,000 square inches, of very large ones up to about 25,000 square inches.

DEGREE OF INFECTION⁶.—*Red currants*.—In the past twenty-five summers of study of the white pine blister-rust from Maine to Minnesota, the writer has never seen what he could term a heavily infected red currant bush. By "heavy infection" is meant the maximum development of the blister-rust upon completely infected leaves of the European black currant, which should be taken as a standard of comparison. While some varieties of red currants are very susceptible (Hahn, 1930, and Spaulding, 1922a) and while there is no reason why some individual bushes of other varieties should not be susceptible to attack, it is a fact that cultivated red currants in general are not heavily infected by the blister-rust fungus (Snell, 1941a and 1941b). What is usually called a "heavily infected" bush is heavily infected *only for red currants*, but never in the writer's experience in the terms defined above. In blister-rust discussions, black currants, wild species of *Ribes* and cultivated currants have subconsciously been considered as belonging to different categories in this respect. It is certain that when red currants have been described as "heavily infected," the describer never intended to convey the impression that they were infected to the degree common on black currants. The same has too often been true also with respect to wild gooseberries.

In 1937 in the counties around Syracuse and Rochester, 26 plots of red currants with a total of 2,928 bushes (2,715 in one plot) were found to be infected as follows: 6 bushes rather heavily infected for red currants (4 per cent to 6 per cent of the entire leaf area); 33 bushes moderately infected for red currants (1 per cent to 4 per cent); the remaining 2,889 only slightly if at all infected. By "slightly infected"

⁶ Throughout this paper, degree of infection of leaves, bushes or lots of bushes is in terms of total discolored area against total leaf area in each case, expressed as percentage.

is meant to the extent of only a few infection spots per bush—5 to 300, or one spot to 50 to 3,000 leaves. The infection percentages of the various plantings ran from 0.0001 per cent to 2 per cent (on a total area basis), with an average of 0.77 per cent for all of them. The average infection a bush was 0.0432 per cent, including the 2,715 bushes in one plot, 0.743 per cent without them.

In the eastern Adirondack region, red currants have been found in 21 gardens, for a total of 343 bushes. Of these bushes 4 have been found heavily infected *for red currants*, 2 moderately infected, and the remaining 337 not at all infected or with only a few spots to the bush. Nine of the plantings were not at all infected and the heaviest infection was 1.17 per cent. The average infection of the plantings was 0.15 per cent and of individual bushes was 0.131 per cent.

While individual leaves may have 75 per cent of their areas covered by blister-rust spots, the most heavily infected bushes in gardens have been from 5.5 per cent to 6.6 per cent infected. The more lightly infected bushes have been 1/4000 of one per cent to 1 per cent or 2 per cent infected. On the most heavily infected garden bush encountered in these studies (6.6 per cent), 0.8 per cent of the leaves were 75 per cent infected, 3.2 per cent were 30 per cent to 50 per cent infected, 13.7 per cent were 10 per cent to 29 per cent infected, 31.5 per cent were 3 per cent to 9 per cent infected, 25.1 per cent were 0.5 per cent to 2 per cent infected, 4.3 per cent had one spot per leaf and 21.4 per cent had not a single spot.

Black currants.—The leaves on bushes of these plants will have up to 97 per cent of their areas infected, with most of the leaves 75 per cent to 95 per cent infected. Of the total leaf area of a bush, between 75 per cent and 80 per cent will be infected and ultimately⁷ bear telia. Furthermore, all of the bushes in a garden will be equally heavily infected. Resistant black currants have been rare in the writer's experience.

Wild gooseberries.—The infection of individual leaves of both prickly and smooth gooseberries has been found to vary from a fraction of one per cent to 77 per cent on ordinary pasture bushes, with relatively few leaves over 25 per cent infected. On small bushes of one or two young shoots, growing in the shade, the amount of infection can be much higher. For example, one "bush" consisting of one shoot 10 inches high growing in a close pine stand had 14 leaves varying in area from 1/20 to 1/3 of a square inch, which were 56 per cent to 100 per cent infected, with a total for the "bush" of 73 per cent infection.

On the most heavily infected sizable bush found in the last few years, individual canes had from 16 per cent to 35 per cent of their leaf areas covered with telia, the average for the bush being 28 per cent.

No exact counts or measurements have been made of all the bushes in a pasture, but on the basis of ac-

⁷ In the west, Kimmey (1938, p. 317) gives 65 per cent of the lower leaf surface infected and 60 per cent bearing telia, as "estimates based on lighter rust infection conditions, some of which gave values as great as those given in the table for this species."

curate data obtained for the leaves of a few bushes, it has been estimated that a fair average for a pasture as a whole would be 15 per cent to 20 per cent of the total leaf area bearing telia under the most favorable conditions of recent years.

FACTORS REDUCING THE PRODUCTION OF SPORIDIA ON RED CURRANTS.—It is seen from the foregoing that while individual leaves of red currant may be about as heavily infected as the leaves of other species, the maximum infection of bushes is comparatively low and for the vast majority of them is very low. Further, even this condition of low susceptibility does not give a true idea of the potential sporidium production, the infecting ability, for there are many factors at work limiting the total amount of infective material from red-currant bushes. These must be emphasized.

Number of infected bushes in a lot.—Whereas the bushes in a garden of black currants will all be uniformly infected, because resistant black currants are very rare, and a pasture of wild gooseberries can be and usually is quite heavily infected throughout, all the bushes in a garden⁸ lot of red currants, as far as has been observed, are never infected to the maximum degree possible for these plants. In fact, the writer has never thus far found a patch of red currant bushes with more than two of the six, or two of the twelve, bushes as much as 4 to 6 per cent infected. More often only one of the six or twelve bushes has been infected to this degree, or none at all (Snell, 1941a). York has also observed this condition in New Hampshire and western New York (letter of March 13, 1939).

High degree of resistance of mature leaves.—Hahn (1930, p. 107) pointed out that red currant leaves which have commenced to harden are not susceptible to infection. York has found the same to be true (same correspondence) and adds that if the leaves do become infected during the late hardening process, no uredia or telia are developed.

In a thorough study of three lots of red currants (one of them reported upon in detail elsewhere (Snell, 1941a)), the writer found that infection spots of late June and early July were 0.005 to 0.05 sq. in. in area, averaging 0.0112, while infection spots from the 10th of July on were rarely 0.005 sq. in. in area, were mostly punctate, with a single uredium, and averaged 0.0038 sq. in. in area. Over 100,000 infection spots were measured in this study.

Tendency of red currants to produce a single set of leaves per season.—Hahn (1928, p. 668) has noted the normal tendency of red currants to produce only a single set of leaves in the spring, and the absence of young leaves during the summer, contrary to the habits of wild gooseberries and *R. nigrum* (cf. Metcalf, 1924). In the writer's experience this is ordinarily true, but not universally so. While uninfected,

⁸ The words "in a garden" should be noted in this connection, because this generalization does not hold for planted red currants that have been abandoned and are allowed to grow uncared for (Snell, 1941a). The latter are almost always, if not always, more heavily infected than those in a well-cultivated patch.

lightly infected or moderately infected bushes put out no new leaves, the worst infected bushes will, on the other hand, sprout new shoots (Snell, 1941a).

Lowered viability of teliospores and sporidia.—Spaulding stated (1922a, p. 24) that resistant species and varieties produce a decreased number of uredia and telia and that the telia produced have a lowered viability. He also showed (1922b, p. 222) that telia on necrotic spots on *R. nigrum*, *cynosbati* and *rotundifolium* are not germinable. It is extremely likely that the same is true for *R. sativum*. York has observed that after the leaves attain a certain age, if uredia and telia are formed, they are poorly developed, have a low germination capacity and weak germ tubes or promycelia (same correspondence). Here again the writer has no new data of his own available for purposes of the treatment which follows, but the facts should be kept in mind when one considers the comparative numbers of sporidia produced by different species of *Ribes*.

Other important factors.—There are three other factors acting to reduce the total production of sporidia upon red currants which are probably the most effective of all. They are: (1) the early defoliation of red currant bushes as compared with other species of *Ribes*; (2) the necrosis of blister-rust spots; and (3) the reduced size of telia upon infection spots, whether the spots ultimately die or not. Some idea of the possibly very great, if not critical, importance of these factors may be obtained from the following brief statements: (1a) infected red currant bushes drop their leaves earlier than uninfected bushes; the more heavily infected they are, the earlier this occurs, with the most heavily infected leaves falling first; (1b) while infected red currant bushes may not be entirely defoliated until along in October, on the other hand most of the infected leaves are dropped before the middle of August; (2) in two patches of abandoned currants, the necrosis of spots was 75 per cent and 96 per cent before August first in 1940; (3) in these same two patches, 95 per cent of the telia were only 22 per cent to 50 per cent of the normal length. A complete discussion of these factors is given elsewhere (Snell, 1941a).

Recapitulation.—Even though at present precise or even general quantitative evaluation of the effect of these factors in reducing sporidium production on red currants is for the most part, if not entirely, infeasible or impossible, it is not difficult to realize that their several or total effects may be important or even decisive.

By way of further re-emphasizing these factors, it may be well to reproduce a quotation from Spaulding (1922b), which forcefully presents the situation in a negative manner. He says that *Ribes nigrum* "is dangerous as compared with other species because: (1) the plant is nearly maximum in height. (2) It is maximum in vigor of growth. (3) It produces new growth throughout the season. (4) It produces new shoots and leaves to a maximum lateness in the season. (5) It produces a maximum area of leaf surface. (6) It is more susceptible than any other species.

TABLE 1. Pathological specifications of comparable units of three species of *Ribes* and potential production of sporidia.

	1	2	3	4	5	6	7	8
	No. bushes	No. leaves	Area of leaves in sq. in.	% Infection	Area infected leaf surface (sq. in.)	Total no. telia for the lot	Total no. sporidia for the lot (in millions)	Rough ratio of nos. of sporidia in column 7
Black currants	6	30,000	124,080	77%	95,542	382,168,000	2,293,008	70
Prickly gooseberries	28	93,000	67,890	20%	13,578	44,128,500	264,771	8
Red currants	6	30,000	100,380	2.33%	2,340	5,616,000	33,699.6	1

(7) A maximum number of telia per unit of leaf area are produced upon this host. (8) These telia are of maximum vigor in germination and production of sporidia. (9) As shown by the above investigations, the telia produced on this host are of maximum longevity in the season."

POTENTIAL SPORIDIUM PRODUCTION OF COMPARABLE UNITS OF THREE SPECIES OF RIBES.—From the foregoing measurements of leaf areas and from counts of telia, and with a consideration of the only limiting factor at present susceptible of reasonably accurate evaluation for a general discussion, a determination of potential sporidium production has been calculated for comparable units of black currants, prickly gooseberries and red currants (table 1). Suppose that we take as these comparable units a row of six black currants, six red currants and the pasture half acre of prickly gooseberries discussed above using the data presented in the preceding sections of this paper.

The six black currants and the six reds (column 1) are certainly comparable, for these bushes are usually planted in sixes or dozens. The selection of something comparable in the gooseberries was difficult, because gooseberries occur scattered in fields and pastures, and as a rule not in clumps. There is of course no method at present known for determining the equivalent of the scattering or segregation of wild gooseberries in terms of concentration of cultivated *Ribes* (of bushes and hence of sporidia). It was thought, however, that the pasture half acre of gooseberries discussed above was a fair representation for our purposes, because the lack of concentration of sporidia characteristic of the cultivated bushes would be roughly equalled by the greater number of wild bushes. Further, this lot of gooseberries appears to be a fair sample, especially because the pines in the woods on all sides of it were infected to a degree quite typical of such locations in the Adirondacks—roughly 40 per cent bearing blister-rust cankers (Snell, 1928).

As to the number of leaves in column 2, bushes of 5,000 leaves were considered average for the black and red currants, even though in the writer's experience black-currant bushes are on the average larger and more vigorous than the reds [cf. Spaulding, 1922b, p. 224, items (1), (2), (3) and (5)]. The number of gooseberry leaves in the table is the one found by count and estimate.

The figures for area of leaves in column 3 are the result in each case of multiplying the number of leaves by a factor which is the average area obtained from the data discussed previously—for black currants 4.136, for red 3.346. For the gooseberries, the figure was taken directly from the data obtained in the pasture.

The percentages in column 4 have been discussed above. The only variation from those figures is that for the red currants, for which a maximum of 7 per cent was taken as fair, even though the largest percentage thus far actually found was 6.6 per cent. This was divided by three, because as mentioned, no more than two out of six or twelve have been found infected at any time in any garden.

The area of infected leaf surface (column 5) was obviously obtained by multiplying the figures in columns 3 and 4.

The numbers of telia (column 6) were obtained by multiplying the figures in column 5 by a factor found by actual counts of many leaves for each species. The averages in round numbers of telia to the square inch of infected leaf surface, from leaves of all degrees of infection, from different locations and in different seasons have been found to be as follows: black currants, 4,000; wild prickly gooseberries, 3,250; red currants, 2,400. Note that no account is taken of the possibility of a reduced number of telia on red currants, because of necrosis of spots, etc.

No work has been done by the writer on the relative numbers of teliospores or sporidia to a telial column produced by the different species. Taylor's 1,500 teliospores per column (1922) have been accepted as satisfactory for this discussion, and it has been assumed that each teliospore would form 4 sporidia. Hence, the numbers of sporidia in column 7 are obtained by multiplying the figures in column 6 by 6,000. No account has been taken of the factors which may, and usually do, reduce the numbers of sporidia produced by the resistant red currants, such as shortened season, necrosis of spots before telia are produced, short telia, and the reduced germinability of telia (Spaulding, 1922b).

Column 8 gives the rough ratio of the numbers of sporidia produced by the three species, under what are certainly only average or good conditions for black currants and prickly gooseberries, but the maximum conditions found for red currants. If one red currant bush were only 1 per cent infected and

five others were hardly infected, as is often the case, the ratio between the numbers of sporidia produced by the blacks and reds would be nearly 1,000:1. Or if the average infections of the red currants in the Adirondacks in 1936, 1937 and 1938, and in the western counties in 1937 were used, the ratios would be about 300:1; 1,000:1; 1,800:1 and 210:1, respectively. For some of the garden plots having the lowest degrees of infection (for example, one with 0.0001 per cent), the ratio is 1,625,000:1. In the latter case, fewer than four million sporidia would be produced on six red currants.

By a strange coincidence, if 6,600 feet (a mile and a quarter) is taken as a dangerous distance for black currants, a direct proportional calculation gives 750 feet as the dangerous distance for the prickly gooseberries, which accords with the common practise of eradication of *Ribes* other than black currants within 900 feet of pine. A similar calculation would give 95 feet for red currants, which, likewise strangely enough, is about what the writer believes is approximately correct, if not too great.

THE THRESHOLD (OR QUANTUM) RELATIONSHIP BETWEEN SPORIDIUM PRODUCTION AND INFECTION OF PINE.—This study is based upon data derived from damage-studies made in New York State (Snell, 1928, 1931a, and 1931b) and upon data from more recent studies of two species of wild gooseberries growing under different conditions and in different locations.⁹ From the latter data on total leaf area, total infection and potential sporidium production of wild gooseberries, three sets of data were selected (two for *R. cynosbati* and one for *R. rotundifolium*) to be used with the data from each damage-study plot. These three sets of data were used in order to avoid any arbitrary selection of a particular species of *Ribes* or any particular growth and infection conditions of the wild gooseberries, and in order to give some range to the resulting figures. It is true that such calculations will give only rough approximations or indications, because no definite data are available in regard to the wild *Ribes* on the damage-study plots during the period (roughly 1910 to 1923) in which most of the pine infections took place. The numbers and sizes of *Ribes* bushes on and around the plots are, however, known as they existed at the time of the studies and for a few years thereafter in each case. These figures were used in these calculations as if the same numbers of bushes and the same amounts of leaf-bearing stem had been on the plots during the entire period in which the pines were exposed to infection. It was assumed that the *Ribes* had been infected at a maximum throughout the infection period.

With these assumptions and in disregard of any considerations of the vicissitudes in the life-histories of the various *Ribes*-populations, calculations were made of the amount of infected leaf surface and of the possible number of sporidia produced on any

plot over the entire pine-infection period and this number was divided by the total number of permanently successful infections (the number of cankers), in order to obtain some idea of the number of sporidia required to produce a single canker. This final result was considered the threshold number or the quantum for the plot.

It is naturally expected that the figures so obtained would vary greatly, not only because the fundamental data are only approximately accurate, but also because the meteorological and ecological conditions of the plots differ considerably. Even so, the differences in the threshold figures do not vary inordinately, and it appears that they would serve the present purpose.

These threshold figures or sporidial quanta calculated for fifteen of the writer's damage-study plots vary from a low of 580 million to a maximum of 79 billion, with an average of about 12 billion. The calculations for two of the plots gave the extreme figures—from 31 billion to 79 billion—while the figures for the other thirteen plots ranged from 580 million to 26 billion. Since the *Ribes* data on the two plots yielding the extreme figures are not as reliable as those on the other plots, it seems best to eliminate these from consideration. Without them, the average figure is about 5.5 billion.

Without the two plots just mentioned, the lowest figures (580 to 1,200 million) belong to the Kelm Mt. plot (Snell, 1928, 1931a, and 1931b) and the highest (14 to 26 billion) to the McPhillips No. 2 Plot in the Pack Forest. The former, with the highest infection known to the writer in the east (96 per cent), is in a pocket in the mountains where conditions are unusually favorable for infection—frequent fogs, wet foliage at least waist high until noon or 2 P.M. daily during the summer, etc. The latter plot, with the lowest infection of all the plots studied (10 per cent) is the driest. It is an isolated stand of slowly growing pines on an open, sandy plain.

If we now compare these threshold figures with the estimated sporidium production for red currants in table 1—580 million to 26 billion, with 33 billion, respectively—we see that there may be something in the threshold of quantum idea. As the figures stand, there is no stupendous discrepancy between the number of spores necessary for the formation of a single canker over a period of thirteen years and the maximum number of sporidia on red currants. If we confine our attention only to the lowest threshold figure obtained (580 million for the 96 per cent infected Kelm Mt. plot), it is seen that the 33 billion sporidia on red currants annually would produce only 60 cankers per acre. It is not only justifiable, but quite necessary, however, to keep in mind certain facts. In the first place, the sporidium production for garden red currants given in table 1 is high, not only because the percentage of infection used (7 per cent) is higher than any found in these studies, but also because no case was met with in which even two of the six bushes in a row were infected to the maximum degree (6.6 per cent) noted for one

⁹ Snell, Walter H. Report of Investigations of Forest Tree Diseases in New York in 1940. New York Conservation Department, Albany, New York.

bush. In the second place, the sporidium production figure is high, because no account is taken of the possibly very great, if not almost complete, reduction of the sporidial output by such factors as early dropping of leaves, necrosis of spots, decreased production of telia, decreased viability of telia, etc., etc. In the third place, the threshold figures are probably minimal, because these figures are based on the *Ribes* populations extant at the time of study, after ten or fifteen years of deterioration through attack by blister rust, by other diseases and insects, through shading as the stand closed, etc. (cf. Pennington, 1922). Only moderate changes either way would bring the potential production of sporidia on red currants quite close to the minimum threshold figure, and quite likely, below an average threshold figure, not to mention the maximum figures. Further, it is evident from the discussions given here and from the data upon the Crown Point Center red currants (Snell, 1941a), that under certain conditions a very insignificant volume of sporidia would be produced—a volume of sporidia too insignificant to be of any danger to white pines at any distance from them. Indeed, it is quite possible that under extreme conditions, infected red currant bushes would produce no sporidia at all.

DISCUSSION AND CONCLUSIONS.—The study of infection of pines growing near red currants (Snell, 1941b) showed that pines are infected only rarely even at short distances from the bushes. The evidence presented in this paper shows why the foregoing situation prevails.

From the data presented upon the volume of sporidia produced, it is shown that there is a great discrepancy between the infecting powers of black currants, wild gooseberries, and red currants, even under conditions of maximum sporidium production on the red currants. Other studies of sporidial volume appear to demonstrate the actuality of a threshold or quantum phenomenon with regard to the infection of pine that can be applied to the dissemination of sporidia from all species of *Ribes*, but which has special applicability to the question of the danger of red currants to white pine. It is uncertain how the data upon production of sporidia by red currants can be translated directly into a proper distance from pine at which to eradicate these plants. The threshold or quantum principle is theoretical and the calculations based upon it lack precision. Nevertheless, in spite of the absence of an absolute conclusiveness to the evidence presented, the indication is very clear that sporidia from red currants cannot infect pine as seriously or from as great distances as those from wild gooseberries, if they can infect pine more than rarely at even very short distances, except under conditions unusually favorable for infection or under conditions in which the number of sporidia on red currants may not be greatly reduced from a theoretical maximum.

From a practical point of view, it would seem that the retention of the 900-foot eradication zone for garden red currants near pine can be justified only

upon the presentation of an abundance of sound evidence of the ability of sporidia from red currants to infect white pine at reasonable distances.

SUMMARY

From a considerable body of data upon the number of leaves, total leaf area, number of telia and number of sporidia per bush for cultivated red currants [*Ribes sativum* (Rchb.) Syme], cultivated European black currants (*R. nigrum* L.) and wild gooseberries (*R. cynosbati* L. and *R. rotundifolium* Michx.), it is shown that a garden row of red currants will produce only a fraction of the number of sporidia produced by wild gooseberries and a much smaller fraction of the number produced by black currants, even under maximum infection conditions for the red currants.

Several factors which act to reduce the total sporidium production on red currants are discussed. These are the small number of bushes in a row or patch that become infected, the high degree of resistance of mature leaves, the tendency to produce a single set of leaves a season, lowered viability of teliospores and sporidia produced, early defoliation, necrosis of blister-rust spots, and reduced size of telia.

No attempt is made to reduce the sporidium production figure for red currants to a definite distance from pine at which these plants should be eradicated, but by the use of the "threshold" or "quantum" principle, which derives a certain theoretical volume of sporidia for the production of a single canker on pine, it is demonstrated that maximum sporidium production by red currants in New York is at least very close to, if it may not be far below, the lowest limits necessary for the infection of pine, especially for serious infection, unless possibly under unusually favorable circumstances.

As with previous studies, this study definitely indicates that red currants cannot be considered very dangerous to white pine, if at all so, and that the necessity of applying the 900-foot eradication zone to them in gardens should be conclusively demonstrated.

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EFFECTS OF FLUORESCEIN ON PLANT GROWTH¹

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IN RECENT years many substances have been claimed to increase the growth of plants when added to the soil or nutrient solution. Whenever such a substance is an essential building stone for the plant, not readily obtainable except by uptake through the roots, its effect is not questioned by scientists. When it is a common constituent and is synthesized by the plant itself, such as sugar or vitamin B₁, an effect can be expected only when the synthesis is not sufficient to cover the needs of the plant. In such cases a large amount of work is required to establish the exact conditions under which these substances can increase growth. Provided these conditions are established, scientists are willing also to accept the effectiveness of this second group of substances as growth-promoting.

There exists a third group of substances, which are claimed to increase growth, but which are not found normally in plants or soils. Their effectiveness cannot be deduced from theoretical considerations, unless their effect on cell or organ is already known. Scientists in general are loath to admit the effectiveness of such extraneous substances, unless very extensive experiments have been carried out. It has to be admitted that this attitude is due partly to many claims which have been based on erroneous or insufficient experiments.

To this third group belongs fluorescein, a substance not known to be present in plants normally,

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but which has growth-increasing or -inhibiting properties under certain conditions. The growth-promoting effect of fluorescein has been reported over a number of years by Sellei (1934, 1935, 1936 a and b, 1940, 1941). Private communications from various investigators have seemed to cast doubt on the general effectiveness of fluorescein, as claimed in the above-mentioned papers. Other investigators, however, have observed growth increases after treatment with fluorescein, but have been "hesitant about getting connected with a field which seemed to be in very low repute among biologists." In this case the hypercritical attitude or excessive skepticism of biologists has become a hindrance to progress, since it has prevented the publication of pertinent facts.

Fluorescein has been used by many investigators to study the rate of waterflow in the xylem (e.g., Strasburger, 1891, p. 551). Such experiments were always of short duration, so that no effects on growth were observed. A very interesting use of fluorescein was made by Schumacher (1930, 1933, 1936, 1937) and others to study movements of substances in the phloem of plants. In his first experiments, in which he tried to follow with the fluorescent microscope the translocation of eosin through the conducting elements of the plant, Schumacher (1930) found that eosin specifically plugs the sieve tubes and that it stops all transport through the phloem. Fluorescein was not toxic in this respect. It seemed to have no specific effect on the plants on which it was tested, and apparently it did not prevent translocation. All experiments in which fluorescein was used to study translocation were of relatively short duration (a few days at most), and no effects of the dye on

ARRANGEMENT OF PLOTS

B E E T	Plot Number #3
---	Treated with ---
B E A N	Fluorescein
M A R I G O L D	Root 51
	Top 375
	Total 426
T O M A T O	Root 50
	Top 450
	Fruit 553
	Total 1053



B E E T	Plot Number #4
---	Control ---
B E A N	
M A R I G O L D	Root 32
	Top 231
	Total 263
T O M A T O	Root 42
	Top 364
	Fruit 414
	Total 820

←-----12'-----→

B E E T	Plot Number #2
---	Control ---
B E A N	
M A R I G O L D	Root 38
	Top 244
	Total 282
T O M A T O	Root 89
	Top 550
	Fruit 413
	Total 1052

B E E T	Plot Number #5
---	Treated with ---
B E A N	Fluorescein
M A R I G O L D	Root 58
	Top 282
	Total 340
T O M A T O	Root 60
	Top 484
	Fruit 1066
	Total 1610

B E E T	Plot Number #1
---	Treated with ---
B E A N	Fluorescein
M A R I G O L D	Root 58
	Top 432
	Total 490
T O M A T O	Root 116
	Top 769
	Fruit 583
	Total 1468

B E E T	Plot Number #6
---	Control ---
B E A N	
M A R I G O L D	Root 44
	Top 290
	Total 334
T O M A T O	Root 48
	Top 498
	Fruit 741
	Total 1287

growth were reported in that connection, nor are these experiments helpful in explaining the effect of fluorescein when plants are watered with dilute solutions.

The experiments on the photodynamic action of fluorescent dyes are of more importance for an explanation of the effect of fluorescein on growth. Boas and Merckenschläger (1925) showed that soaking of seeds in eosin causes their roots to become negatively geotropic. Similar observations were made by others; especially interesting is Boas' observation (1933) that eosin abolishes the phototropic response of *Lolium* seedlings. Boysen-Jensen (1934) observed that roots, whose geotropic sensitivity had been abolished by erythrosin, did not further produce auxin. Skoog (1935) found that indoleacetic acid in solution, stable in sunlight, is inactivated by light as soon as small amounts of eosin are added to the solution.

This photodynamic effect is similar to that found in animals, and it is a general property of the group of fluorescent dyes to which eosin belongs. For this reason fluorescein can be expected to have the same effect. Indeed, it has been found that application of strong concentrations of fluorescein to plants causes a typical stunting (Sellei, 1935), which was confirmed again in experiments carried out last summer and in others reported below. It was first believed that this effect could be attributed to the photodynamic destruction of auxin under the influence of fluorescein. This phenomenon cannot, however, explain the growth increase obtained after application of low concentrations of fluorescein.

The present paper is based on data obtained from a series of experiments made at the California Institute of Technology and at the Huntington Gardens and Library, Pasadena, and deals mainly with three problems: (1) establishment of the effects of fluorescein on growth under different soil conditions and with different plants; (2) estimation of the presence of fluorescein in the different parts of treated plants; (3) location of fluorescein effect in the plant.

Field experiments were carried out from May through November, 1941, with fluorescein applied, for the sake of convenience, in the form of 0.33 gm. Photosensin tablets. The formula of Photosensin is the following: fluorescein 5 per cent; iron sulphate 1 per cent; copper sulphate 0.1 per cent; sodium bicarbonate *ad* 100 per cent. The last ingredient hastens the dissolving of the dye and facilitates its handling. The iron added in this way is only one-quarter the concentration supplied by the nutrient solution; the copper is about equal. For this reason the controls and Photosensin treated plots did not differ essentially in their iron and copper supply. Further details of the method of application have been given by Sellei (1941).

FIELD EXPERIMENTS.—One experiment was conducted under semi-practical conditions at the Hunt-

ington gardens in San Marino.² A plot 50 x 120 feet, in light sandy loam, was laid out so that eight irrigation ditches, five feet apart, ran lengthwise through it. Tomatoes (*Lycopersicum esculentum*), corn (*Zea Mays* golden bantam), and eggplants (*Solanum melongena*), were planted along both sides of the irrigation ditches. The field was irrigated by running water through the ditches for about three hours as often as necessary. Seven times, ten to twenty days apart, fluorescein was added to the irrigation water of four ditches in the form of Photosensin tablets; the other ditches were kept as controls with pure irrigation water. The Photosensin tablets (seven to eighteen per treatment per ditch) were dissolved in six liters of water, and this solution was added dropwise to the irrigation water. Some weeks after planting, when growth seemed unsatisfactory, all rows were fertilized with Vigoro. This procedure was repeated a week later.

The experiment was not entirely satisfactory as late planting prevented full development of plants and fruit. The field proved to be inhomogeneous, for growth along the control ditches was uneven. However, an increased yield was indicated, as follows: corn yield of treated plants, an increase in weight of 21 per cent; tomatoes, an increase in fruit weight of 37 per cent; eggplant,³ an increase in fruit weight of 55 per cent. To analyze the results in greater detail a number of well-controlled plot and pot experiments were carried out.

PLOT EXPERIMENTS.—Six plots twelve by twelve feet were laid out in a rectangle, with strips of three feet separating the plots. Figure 1 shows the arrangement of the treated and control plots, which were alternating, to rule out accidental differences in yield due to systematic soil differences. The soil was heavy adobe and the plants received full sunshine. The plots were divided into four equal parts and planted, identically for all plots, with tomatoes, marigolds, beans, and beets. The plants or seeds were spaced one foot apart and were thinned as necessary. All plots were thoroughly cultivated and fertilized twice with ammonium sulphate. Treated plots received fluorescein, in the form of Photosensin tablets in solution. From May 23 until August the solution was given each ten to eighteen days, after which it was repeated every twenty to thirty days.

For the first treatment each of the treated plots received four tablets of Photosensin, with the same amount for the second treatment. Subsequent treatments consisted of five tablets of Photosensin for each of the three plots. Each tablet of 0.33 gm. was dissolved in two gallons of water. This corresponds to one part of fluorescein in 450,000 parts of water.

² The authors wish to thank the trustees of the Huntington Gardens and Library for permission to conduct this experiment, with particular thanks to Mr. W. Hertrich and Mr. M. Gschwind for their help and advice.

³ Due to frost the experiment was concluded before most of these fruits had ripened.

Fig. 1. Ground plan of the plot arrangement of the experiments of tables 1-4, showing the relative location of the plants. For marigold and tomato the net weights per plant in each plot are given in grams.

TABLE 1. Measurement of Marigold plants in plot experiment. Each figure the mean of 38 to 40 plants.

		Weight in grams per plant		Increase due to treatment	
		Treated with fluorescein	Control	Absolute	Per cent
Aug. 6	Weight of whole plant	390.7	306.3	83.8±28.7	27.3
Sept. 26	Weight of whole plant.....	363	256	107 ±34	41.8
	Weight of roots per plant.....	55.8	38.3	44.7
	Number of flowers per plant.....	26.9	19.7	36.5
	Flower surface per plant.....	520.5 cm. ²	209.5 cm. ²	148

After the treatment the plots were watered immediately until the soil was saturated. Controls received the same amount of water. The amount of fluorescein used in this experiment had been found to be optimal in experiments performed in the same type of soil in previous years (Sellei, 1940, 1941).

Experiments with Marigolds (Tagetes erecta).—Two-month-old plants of the "African Lemon" variety were set out May 23, 1941, at one-foot distance. By August 6 they had become crowded, and alternate plants were cut at ground level and weighed. Throughout their flowering period the treated plants showed a greater abundance of blooms. Flowers were cut off twice each month, so that flowering continued for a long time. A difference was apparent in the quality of treated and control blooms, the treated being larger and fuller. To obtain more objective data, actual measurements of number and diameter of flowers were taken September 4 and 18. As a more representative figure for the appearance of the flowering plants the surface instead of the diameter of the flower was calculated. Table 1 indicates that the flowers were much larger and more numerous in the treated plots, agreeing with the results of last year's experiments, as reported by Sellei (1941).

On September 26 the remaining plants were cut at ground level, roots were dug up and both were weighed individually. Table 1 gives the difference in weight between the treated and controls in percentages. It will be seen that the weight per plant was less in this second harvest than in the first, which

occurred just prior to flowering. This can be attributed to the cutting off of flowers and the dying of older leaves.

The arrangement of the marigolds in plots is shown in figure 1. It is evident that those in each of the treated plots were better than those in the adjoining control plots. However, the results varied within certain limits in the three treated plots as well as in the controls. Probably the arrangement of the plots helped to overcome existing soil differences. The difference in quality as expressed by flower surface was over 100 per cent in favor of the treated.

As a result of the statistical evaluation of plants harvested August 8 and September 26, it was found that the probability of the occurrence of the mean treated in sampling from a homogeneous untreated population was in each instance about three chances in one thousand. Considering, however, that both results were obtained from the same plots for the same plants at an earlier and a later date, the chances in the second harvest were still much less. This repetition of occurrence in one experiment considerably increases the value of the statistical results obtained.

Experiment with tomatoes (Lycopersicum esculentum).—Tomato plants of the Stone variety were set out May 23, 1941. The treatment was identical with that of the marigolds. Plants were thinned, leaving forty-four plants for treatment and forty-five for controls. All plants were staked and numbered. Ripe tomatoes were gathered from August 28, with a total of nine harvests to October 21, when the last

TABLE 2. Effect of Photosensin on tomatoes in plot experiment.

	Treated Control				
Number of plants.....	44	45			
Number of tomatoes harvested.....	491	427			
	Total weight in grams of all plots		Weight per plant in grams		Increase after Photosensin treatment in per cent
			Treated	Control	
Fruit production	32,459	23,531	737.7	523	41
Weight of tomatoes harvested—per fruit	66.1	55.1	20
Weight of whole tomato plant.....	24,762	21,203	562	471	19
Weight of roots	3,277	2,698	74.5	59.9	24
Total sugar content of fruit.....	3.93%	2.87%	36.5
Sucrose content of fruit.....	1.70%	0.40%	Over 100

TABLE 3. *Effect of Photosensin treatment on bean plants in plot experiment. Total of three treated and three control plots. Number of plants: 88 treated; 90 control.*

	Weight in grams, total of all plants		Weight per plant in grams		Increase after Photosensin treatment in per cent
	Treated	Control	Treated	Control	
Yield of beans on July 30.....	2,464	1,825	28	20.3	38
Yield of beans on August 19.....	933	612	10.6	6.8	56
Yield of both harvests per plant.....	38.6	27.1	42.5
Weight of plants in grams.....	8,620	7,446	97.9	82.7	18.3

was made. On that date the tops of plants were cut at ground level, roots were dug up, and *all* were weighed. At all harvests the crop of each plant was collected separately and weighed individually, and the weights for each plant were totaled at the conclusion of the experiment. Tomatoes from each plant also were counted and the sugar content was determined. Leaves of the plants began to turn brown in October and the plants were in a state of deterioration when harvested October 21. Table 2 gives the pertinent data of this tomato experiment. The total crop was statistically evaluated and the difference of means was found to be:

$$\Delta = 214.8 \text{ grams} \pm 89.2 \text{ grams}$$

showing that the result of the fluorescein treatment was significant so far as the total fruit weight per plant was concerned.

The data presented in table 2 show an increase in quantity as well as an improvement of quality after the dye treatment. The treated plants had a heavier crop through the production of more and larger tomatoes than in the controls. Both treated plants and roots were heavier than the untreated ones. The quality improvement was apparent in the larger fruits with a higher sugar content. The increase was due to the sucrose percentage, which accounts for the sweeter taste of treated fruit. The root systems of the treated plants were more branched and seemed to have a much larger absorbing area.

Experiment with beans (Phaseolus vulgaris).—Black wax beans were sown May 23, 1941, three beans per hill, which were one foot apart. After germination they were thinned to one plant per hill. Flowering came soon and beans were ready for harvest July 30. Only ripe string beans were harvested; the pods which were too young were left on the stems for another harvest, August 19. The har-

vest of July 30 was statistically evaluated. August 19 all plants were removed from the ground and weighed. Data are presented in table 3.

The yield of treated plants was 534 pods, with 460 pods from controls. The weight of the average single pod from controls at the first harvest was 3.9 grams; the weight of the average single pod from treated plants of the same harvest was 4.6 grams, an increase of 17 per cent. The statistical evaluation of the yield of the same harvest showed that the difference between the treated and controls was highly significant as

$$\Delta = 7.7 \text{ grams} \pm 2.7 \text{ grams}$$

As a result of the dye treatment, beans produced a heavier crop with larger single pods, indicating an improvement of quality. The bean plants, also, were heavier in the treated plots.

Experiments with beets (Beta vulgaris rubra).—Beet seeds of Crosby's Egyptian variety were set May 24, 1941. All beets were harvested September 12, no previous harvesting or measurement having been made. For data see table 4. The statistical evaluation of the difference between the treated and control whole beet plant showed that the result of the dye treatment was significant:

$$\Delta = 36.5 \text{ grams} \pm 17.3 \text{ grams.}$$

SANDCULTURE EXPERIMENT.—In this experiment tomatoes and marigolds were grown individually in gallon cans covered inside and outside with black tar paint. Cans were 18 cm. in height and 15 cm. in diameter. In the open ground, the required space was dug up and filled with a two-inch sand and gravel layer on which the cans were placed. The space between the cans was filled with soil. The cans were filled with washed river sand.

TABLE 4. *Effect of Photosensin treatment of beet plants. Number of plants: 61 treated; 63 control.*

	Total weight in grams of all plots		Weight per plant in grams		Increase after Photosensin treatment in per cent
	Treated	Control	Treated	Control	
Weight of whole beet plant.....	12,999	11,123	213.1	176.6	20
Weight of beet roots only.....	8,898	7,934	147.4	125.9	17
Weight per single top.....	65.7	50.7	29.8

Each variety of plant to be tested was set in fifty cans, making ten series in which five and five received the same treatment. Series were numbered from 1 to 10, in which 1 and 10 were controls, receiving Hoagland nutrient solution only, while series 2 to 8 were treated with the same nutrient solution to which different Photosensin concentrations were added. The fifty cans were placed in the ground in two plots with consecutive numbers. The cans of series #1 (controls) were between treated pots in five different places, while all pots of control #10 were at the end of one plot.

Series 1—Control, plain nutrient solution.

Series 2—1 gm. Photosensin in 20 liters of nutrient solution or 1:400,000 fluorescein.

Series 3—1 gm. Photosensin in 30 liters of nutrient solution or 1:600,000 fluorescein.

Series 4—1 gm. Photosensin in 50 liters of nutrient solution or 1:1,000,000 fluorescein.

Series 5—1 gm. Photosensin in 100 liters of nutrient solution or 1:2,000,000 fluorescein.

Series 6—1 gm. Photosensin in 200 liters of nutrient solution or 1:4,000,000 fluorescein.

Series 7—1 gm. Photosensin in 500 liters of nutrient solution or 1:10,000,000 fluorescein.

Series 8—1 gm. Photosensin in 1,000 liters of nutrient solution or 1:20,000,000 fluorescein.

Series 9—1 gm. Photosensin in 25 liters of nutrient solution or 1:500,000 fluorescein.

Series 10—Control, plain nutrient solution.

Method of treatment.—Plants were watered daily with the designated solution. Series 2, 3, 4, 5, 6, 7 and 8 received Photosensin treatment daily; series 9, each fourteen days, only nutrient solution being given on the other thirteen days. Plants of control series were always watered first. The amount used (about 200 cc. for each can) was enough to fill cans entirely with solution and to wash out solution which was left over from the day before. The solution used dripped out of holes in the bottoms of the cans into the sand-gravel layer.

To break off roots which might have grown out of the cans, and to avoid all possible communication between the various plants, the cans were twisted once weekly. Cans were in full sunshine in warm, summer weather, although many of the mornings were heav-

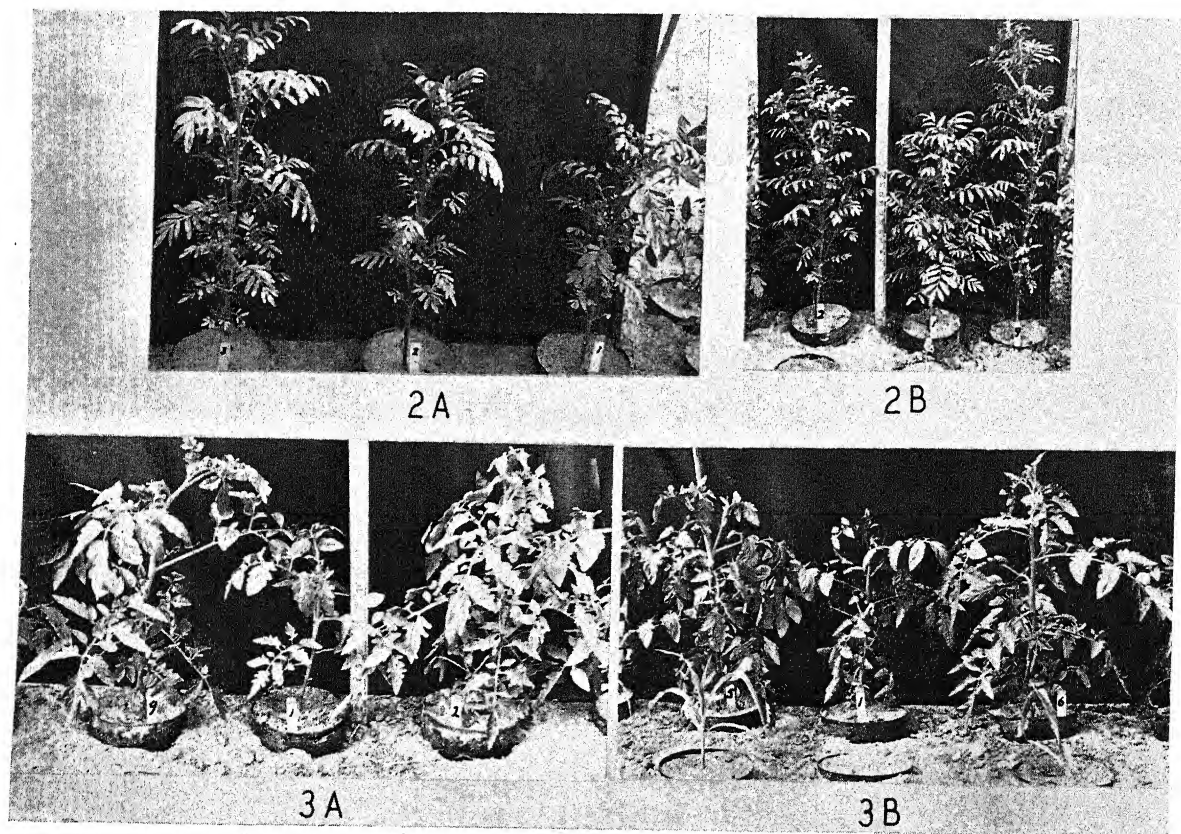


Fig. 2-3.—Fig. 2A. Marigold plants photographed August 12, 1941, twenty-five days after transplanting in one-gallon cans filled with sand. On right: Control (series 1). On left: Two plants watered with fluorescein (series 3 and 2).—Fig. 2B. Plants from the same experiment photographed two weeks later: Center, control; right, series 9; left, series 2.—Fig. 3A. Tomato plants grown in one-gallon cans in sand. Photo taken on August 12, 1941, twenty-five days after transplanting into cans. Middle: Control plant (series 1). Left: Series 9. Right: Series 2.—Fig. 3B. Another control plant in middle; at right, series 6; at left, series 5.

TABLE 5. Development of *Tagetes* plants in the sandculture experiment. For each series the mean is given.

Series:	1	2	3	4	5	6	7	8	9	10
Whole plant g.....	167.2	164	167.2	159.6	192.4	177	190	191.2	192.4	179.8
Roots g.	19.6	24.4	24.2	19.8	30	22.4	28.4	26.4	33.6	20.2
Flowers per 5 plants.....	4	6	9	13	5	7	8	2	9	3
Flower surface in cm. ² per plant	17	30	33	42	20	33	47	10	32	10

ily overcast, and about four days were without any sunshine. In some of the cans two plants were placed. Approximately two weeks later, before they could have influenced each other's growth, one plant was taken out for further testing.

Plants were measured for growth response by taking their height. This part of the experiment had to be considered finished on August 20, as the plants were growing rapidly and the cans proved to be too small. Furthermore, the close planting, especially in the case of the far more developed treated ones, allowed no chance to make further vigorous growth. Plants were also weighed, and in the marigolds the flowering was noted, taking into account the time when flowering started as well as the size of treated

best growth response, had a root system 68 per cent heavier than the controls. The earlier flowering, the increased size of flowers and the heavier root system indicate the action of fluorescein.

Experiment with tomatoes (Lycopersicum esculentum).—Two-month-old plants of the Stone variety were set out in cans July 18, 1941. Treatment and arrangement of the cans was the same as in the marigold experiment. Plants of fairly equal size were selected. Differences in growth, foliage and height early became apparent. Figure 3 illustrates some of the plants on August 12. The cans were photographed as they were set in the ground, the pictures including various controls and adjoining treated plants.

TABLE 6. Weight in g. of tomato plants in sandculture experiment. Each figure is the average of five plants.

Series	1	2	3	4	5	6	7	8	9	10
Whole plant	159.3	200.4	210.5	227	207.2	188.8	237.8	229	173.2	184
Root	20.5	31.4	26.4	26.3	27.9	24	29.5	21.2	25.4	18.1

and control blooms. The accompanying photographs illustrate the difference in height and size of control plants as they were placed in the ground, comparing controls with adjoining treated plants (fig. 2, 3).

Experiment with marigolds (Tagetes erecta).—Plants one and one-half months old of the "African Orange" variety were set in cans July 18, 1941, and treated as previously described. Differences in growth, foliage and height early became apparent. Treated plants started to set flowers earlier than did the controls. Plants were tested as to blooms September 12 and 19. On September 19 the experiment was concluded. All blooms were counted, and diameter of blooms measured. Plants were taken out of cans, roots were washed, and tops and roots were weighed (see table 5).

Considering the weight of the plants, the effect of the dye was apparent mostly in the root system. The highest weights were obtained in series 9 with a mean of 33.6 grams, whereas the mean of the two control series was 19.9 grams. Averaging the results from group 5-9 on, the mean root weight was 28.1 grams, showing an increase of 41 per cent. Averaging results from all the treated series, the mean root weight was 26.1 grams, and the increase was 31 per cent.

The harvest of marigold plants showed a considerable increase in the root system of the plants after Photosensin treatment. Series 9, which showed the

As seen in table 6, the treated series had heavier roots in all of the various series, and heavier tops in most series. The mean root weight for both controls was 19.3 grams; the mean of all series treated was 26.5 grams; increase after Photosensin treatment was 37 per cent. The mean of whole plants in both control series was 171.6 grams; increase of whole plant when averaging all treated series, 22 per cent. Therefore the harvest on September 2, when the experiment was concluded, showed an increase in the weight of the treated plants, especially in their root system.

It is interesting to compare the effect of fluorescein treatment in the sandculture experiments with those of the plot experiments carried out in heavy adobe. Table 7 shows an excellent agreement between the

TABLE 7. Comparison of sandculture and soil experiments. Increase in per cent of all plants treated with fluorescein when compared with the controls.

		Sand culture	Soil experiment
Tomato	Weight of whole plant...	22%	19%
	Weight of root system...	37%	24%
Marigold	Weight of root system...	31%	44%
	Flower surface	120%	148%

two sets of results, indicating that the effect of fluorescein on growth is general and not restricted to one kind of soil.

ESTIMATION OF FLUORESCHEIN IN TREATED PLANTS.

—The foregoing experiments establish the growth-promoting effect of fluorescein when used in low concentrations to water plants. The following determinations were carried out to determine in which manner this effect could be brought about.

In the *Avena* test there is no trace of growth-promoting effect of fluorescein. When agar blocks containing 1:1,000 to 1:100,000,000 fluorescein are placed unilaterally on the cut surface of decapitated coleoptiles, the plants remain perfectly straight. Nevertheless, the dye has penetrated the coleoptile cells. No toxic effects were noticed after twenty-four hours even in the highest concentrations. This shows that fluorescein does not have an auxin-like growth-promoting effect.

A number of determinations of the fluorescein content were made to find its distribution in the treated plant. To this end the plants were extracted both fresh and dried. The material was boiled for a short time with very dilute NaOH or NH_4OH . Small amounts of a fluorescent material were thus extracted from plants treated with 1:400,000 fluorescein, but these amounts were of the same order of magnitude as found in extracts from control plants.

After this negative result the bleeding exudate of tomatoes was collected and investigated as to fluorescein content. The control plants gave practically no fluorescence in their exudate. When the tomatoes had been watered with concentrations ranging from 1:400,000 to 1:2,000,000 fluorescein one to four days previous to collecting the exudate, no increased fluorescence of the exudate was observed. Since the method of determination is sensitive down to a concentration of 1:20,000,000, it follows that the fluorescein is excluded from the xylem during the water uptake by its roots, since the external concentrations were at least ten to fifty times higher than those in the xylem.

The two last mentioned facts make it rather certain that most of the applied fluorescein does not reach the top of the plant. If it were taken up, we have to assume that either its fluorescence is covered, which does not seem very likely, or that the fluorescein is chemically changed so as to give no fluorescence.

The determinations of the fluorescein content of treated roots bear out the above conclusions. Whereas the color of the control root systems was white, the roots of plants treated with 1:400,000 fluorescein were rust-colored, grading down to light yellow in the lower concentrations. These roots were washed free from the adhering sand and were dried and extracted with cold water. The extract was strongly fluorescent. It is difficult to give exact data, since the first washing of the roots may already have removed a considerable amount of absorbed dye, but the determinations show that the fluorescein on the roots was at least five times more concentrated than in the

solution with which the plants were treated. This confirms the earlier conclusion that the root system excludes the applied fluorescein from the above-ground portion of the plant.

When much higher concentrations of fluorescein are applied to the plants by watering them with a 0.3 per cent fluorescein solution, then a marked dwarfing of all parts of the plant results. In those plants, however, fluorescein moves upward in the shoot, which in some cases becomes clearly stained, and sometimes fluorescein is found in the bleeding exudate of such plants.

However, when a 0.2 or 0.1 per cent fluorescein solution is applied, less dye is found in the above-ground parts. Only when the root system is injured do large amounts of fluorescein move up into the top of the plant, which consequently dies. But as long as the root system remains healthy, the top of the plant remains alive also, even in these high concentrations of fluorescein. Only the dead root cells become stained. Small amounts of fluorescein move up in the xylem and are absorbed on the vessel walls, so that in the top of the plant only very small amounts of fluorescein can be detected by extraction. In three cases, in which tops or leaves of plants watered with a 0.1 per cent solution of fluorescein were extracted three days after treatment, less than 0.0001 per cent was detected. Only in one killed plant was a concentration between 0.01 and 0.1 per cent found. In the bleeding exudate of the plants receiving 0.1 and 0.01 per cent and weaker solutions of fluorescein, no trace was detected. Thus, we must conclude that a sort of negative accumulation of fluorescein occurs in the roots.

Greenhouse experiments on the immediate effect of fluorescein on growth.—To study in greater detail the way in which fluorescein exerts its growth-promoting and growth-inhibiting effect, the following experiments were carried out with tomato plants. In an attempt to ascertain whether we were dealing with a photodynamic effect, the growth of tomato plants in darkness was compared with their growth in light, both with and without fluorescein. Since growth of tomatoes stops in darkness within twenty-four hours, due to a lack of sugar, plants in darkness and in light can be made comparable by treating both groups with a 10 per cent sucrose solution. Under such a treatment growth in darkness continues at essentially the normal rate for many days, and the groups growing in light and in darkness have comparable growth rates.

For the following experiments small tomato plants, grown in sand in four-inch pots and watered with complete Hoagland nutrient solution, were divided into two groups. One group was taken from a greenhouse into a darkroom, kept at 27°C . and ± 70 per cent humidity. The other group was moved into an air-conditioned greenhouse maintained at 26.5°C . and 70 per cent humidity. The only difference between the two groups of plants was that during ten hours each day the tomatoes in the greenhouse were exposed to diffuse daylight. All leaves were cut off the

TABLE 8. *Elongation in mm. of main stem and young leaf (10 to 15 mm. long) of tomato plants in the three-day period following watering with fluorescein solutions. Only two full-grown leaves left per plant, immersed in 10 per cent sucrose solution (exp. 1, 2), or left free in air (exp. 3). Each figure mean of six to eight plants. Temperature 27°C.; 70 per cent humidity.*

Concentration of fluorescein	Experiment 1				Experiment 2				Experiment 3	
	Darkroom		Greenhouse		Darkroom		Greenhouse		Greenhouse No sugar	
	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
1:500	5.0	4.2	1.7	26.7
1:1,000	4.0	7.5	3.2	33.3	8.6±1.7	8.5	6.4±1.3	24.8	1.8±0.6	13.0
1:10,000	14.0±0.9	35.0
1:100,000	16.4±0.9	35.4
1:1,000,000	31.5±2.2	8.0	13.9±0.9	30.8	8.8±0.9	24.6
Control, no fluorescein.	14.0	6.8	16.9	39.7	26.9±2.1	8.4	14.4±1.2	36.6	4.3±0.7	19.3

plants except the two youngest full-grown leaves and the leaf primordia less than fifteen mm. long. In most of the experiments the two leaves left on the plants were immersed in glass vials filled with a 10 per cent sucrose solution, so that they could take up the sugar by diffusion through their leaf surface. Part of the plants were watered with tap water and part with different concentrations of Merck fluorescein solutions. Every twenty-four hours the length of the stem and of the largest leaf primordium was measured. The growth of the young leaves in darkness fell off rapidly, so that their growth stopped after two or three days, but stem elongation both in darkness and light, and leaf growth in light, continued for more than four days. After this time the leaves in the sugar solution were mostly injured, so that the conclusions based on longer periods of observation became unreliable.

From table 8 it appears that growth in length of stem in darkness is equal to, or exceeds, that in light, provided sucrose is supplied to the plants. The effect of fluorescein in high concentration is to reduce growth in length 60 to 90 per cent, both in light and in darkness. Consequently, the growth inhibition is not due to a photodynamic effect of the fluorescein, as might have been expected from Skoog's observation (1935) that eosin is a photosensitizer for auxin destruction. The effect of high concentration of fluorescein on leaf growth is much less pronounced, and amounts to 30 per cent growth decrease at most. In another experiment it was found that at 1:2,000 fluorescein there was the same inhibition of stem growth as in the higher concentrations, but no decrease at all in leaf growth. In experiment 2 of table 8 a slight increase in stem growth is observed in the lower fluorescein concentrations. In experiment 3 this effect is more pronounced. Thus, it seems possible to duplicate the growth-promoting effects observed in field experiments in greenhouse experiments of short duration.

DISCUSSION.—The experiments reported in this paper confirm the results of earlier experiments in which fluorescein was found to increase growth when applied in low concentrations and inhibit growth in high concentrations. The growth increase was observed in different plants and under different soil

conditions. The experiments with sand cultures are of special importance, since they show that it is the actual concentration of the fluorescein which is effective. There was little, if any, adsorption of the dye to the sand, which was washed clean every day with a fresh nutrient solution containing fluorescein. Most of the dye was found adsorbed on the root system; actual fluorescein determinations showed that the highest concentration was found around the root system, whereas little or nothing moved up in the shoot of the plant. We have, therefore, to look for the primary effect of the fluorescein in the roots of the plant, since only there are measurable amounts found. This is substantiated by the greenhouse and darkroom experiments. The immediate effect of the applied dye was on stem growth and not on leaf growth, and the effect was the same in darkness as in light. Leaf growth is regulated by growth factors formed in the older leaves and transported towards the young growing leaves (Bonner and Haagen-Smit, 1939). When no fluorescein moves up in the shoot, no direct effects on leaf growth could be expected. Stem growth, on the other hand, is regulated by auxin and caulocaline (Went, 1938). Since the effect is the same in light as in darkness, and fluorescent dyes are known to act as photosensitizers in auxin destruction by light (Skoog, 1935), it is not likely that the effect of fluorescein is on the auxin. Caulocaline, on the other hand, is formed in the roots, and, therefore, could be affected by fluorescein in contact with the root system. Also, no effect of light would be expected if the dye should affect caulocaline production in the roots.

Although no conclusive experiments have been performed, proving beyond doubt the way in which fluorescein increases or decreases growth, all observations are consistent with the assumption that it acts by way of the root system. Since we know that all parts of a plant are interconnected by means of hormones or food correlations, effects on one part of the plant will have systemic effects in experiments of longer duration. In most of the experiments described in this paper the growth increase due to fluorescein was relatively greater in the root system than in the tops, indicating that the effect on the root sys-

tem was the primary and the effect on the top a secondary effect of fluorescein.

The plot experiments in soil were carried out in the heavy adobe of the same type as used in previous years (Sellei, 1940, 1941), so that without preliminary trials the optimal concentration of fluorescein could be applied. This concentration was slightly lower than the one used in earlier experiments in Hungary and may vary from soil to soil. For this reason it is interesting that in washed sand the same effect of fluorescein can be obtained. This makes it possible to carry out comparable experiments in many different localities. It should be pointed out that a repetition of the sand-culture experiment in late fall, when the temperature was much lower and growth of tomatoes was very slow, gave smaller growth increases with the same concentrations of fluorescein which had given good results in the summer months. This is in accord with the general experience, that fluorescein treatment is much more effective during midsummer than at other times of the year. To judge from experience, this seems to be due to the higher temperature and better growing conditions in summer. It should be emphasized that, if our assumption is true, the primary effect of fluorescein is on the hormone production of roots, increased growth can be expected only when all other growth factors—mineral nutrition, assimilation, auxin, etc.—are optimal or at least not limiting growth.

The growth effects of dyes are not limited to those

of the fluorescein group. This observation was made by Sellei in earlier papers (1934, 1935), and more recently by Patterson (1941, 1942), who published data concerning the effect of neutral red on cell division in roots.

SUMMARY

The experiments reported confirm the results of earlier experiments in which fluorescein was found to increase growth of plants when applied in low concentration and to inhibit growth when applied in high concentration.

Plants treated with low concentrations responded by producing heavier tops, roots, more fruit and also by showing a qualitative improvement.

Experiments in sand culture showed practically the same per cent increase as those carried out in plots in heavy adobe, indicating that the effect of fluorescein is general and not restricted to one kind of soil.

Sand culture experiments of short duration in the greenhouse and the darkroom, when giving to young tomato plants fluorescein of high and low concentration, showed that a definite acceleration or inhibition of stem elongation was evident within three days.

A possible explanation is discussed of the mechanism of the action of fluorescein.

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ANATOMICAL AND TAXONOMIC APPROACHES TO SUBGENERIC SEGREGATION IN AMERICAN QUERCUS¹

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BEFORE THE turn of the century it was not uncommon for a taxonomist to take as his primary objective the discovery of relationships within large categories of plants and to use as his principal tool the anatomy of the groups on the assumption that it could be utilized as a key to phylogeny. Most recent taxonomic studies have tended to concern themselves with relationships among lesser taxonomic entities which scarcely demand an anatomical approach.

The purpose of this paper is to bring together the several treatments of subgeneric groups in American *Quercus* which have been based variously on floral morphology and on wood anatomy, to present additional data particularly on the wood anatomy, and to evaluate the several types of data for purposes of establishing the boundary lines between the various groups. The age-old human failing of regarding one's special line of interest as the most important evidence is met here freely. Most workers have conscientiously weighed all the evidence available to them and have rendered unbiased conclusions. Even they, however, often seem not to have realized that the characters they so unconditionally described should in most cases have been preceded by the word "usually." Trelease (1924) is outstanding among them in his readiness to point out exceptions.

Oersted (1871) applied floral and fruiting characters in the genus *Quercus* to effect segregation of the species into subgenera. His subgenus *Erythrobalanus* is characterized by elongate styles with spatulate stigmas, acorn shells tomentose within, and abortive ovules apical. The subgenus *Lepidobalanus* is distinguished by short styles with broad abruptly dilated stigmas, acorn shells glabrous within, and abortive ovules basal. Subsequent observations have added lesser differences between these groups. Oersted's subgenus *Macrobalanus* includes a group of large-fruited species which differ from *Lepidobalanus* in their unequal cotyledons and lateral radicles.

The leaves of *Erythrobalanus*, if at all toothed, lobed, or acute at the apex, have the teeth or lobes and the apex aristate-tipped by an extension of the veins. Those of *Lepidobalanus* fall into two classes. In one the lobing is broadly rounded and lacks any tips; in the other, if the leaves are toothed, the teeth are tipped by short mucronate projections which are very thick with much sclerenchymatous tissue. In no case are the leaves of *Lepidobalanus* aristate-tipped, but in *Q. dumosa* and *Q. turbinella* the mucronate tips may be quite elongate so as to simulate aristae of *Erythrobalanus* to a certain extent. The cup scales of *Erythrobalanus* are characteristically thin, while those of *Lepidobalanus* are corky-thickened basally. The bark of *Erythrobalanus* is hard, smooth, or furrowed (but scarcely scaly) and dark brown or black. In *Lepidobalanus* it is rather soft

and corky, scaly or flaky, and gray or glaucous. In a few species of *Lepidobalanus*, e.g., the series *Virentes*, the bark simulates that of *Erythrobalanus*.

Schwarz (1936) has elevated *Erythrobalanus* and *Macrobalanus* to generic rank on the basis of these same characters. He retains *Lepidobalanus* as a subgenus of *Quercus*, but he proposes *Sclerophyllodrys* as a new subgenus to include, among other groups, what he calls the section *Protobalanus* Trelease, apparently without regard for the fact that Trelease's name was published as a subgenus (Trelease, 1924) and, therefore, antedates Schwarz' name. Trelease, on the other hand, maintains Engelmänn's subgeneric name, *Leucobalanus* (Engelmänn, 1876), even though it is clearly synonymous with *Lepidobalanus* (*sensu* Oersted). Most American writers to date have followed Trelease in his adoption of *Leucobalanus*. Camus (1938) has set up the subgenus *Euquercus* in the sense in which the name *Lepidobalanus* was first used by Endlicher (1847), namely, to designate all those species of *Quercus* exclusive of certain Asiatic species with cup scales fused into rings. Bailey (1910) proposed the subgenus *Biotobalanus* to include the live oaks, a very unnatural group of evergreen species belonging to both *Erythrobalanus* and *Lepidobalanus*. He based this segregation solely on characters of wood anatomy.

The subgeneric names of American oaks, then, are *Leucobalanus*, *Sclerophyllodrys*, and *Euquercus* (which are superfluous names), *Biotobalanus* and *Macrobalanus* (which are scarcely worthy of subgeneric distinction), and *Lepidobalanus*, *Protobalanus*, and *Erythrobalanus*. The question as to whether any or all of these deserve generic rank is not within the province of this paper, but the evidence would seem clearly to prohibit any such splitting (Muller, 1942).

REVIEW OF ANATOMICAL LITERATURE.—Although oak wood was one of the earliest subjects of anatomical investigation (Malpighi, Grew, Leeuwenhoek, and others), Abromeit (1884) was the first to make extensive use of anatomical characters to show relationships within the genus *Quercus*. Investigating the structure of fifty-five species of European, Asiatic, and American oaks, he separated them, on the basis of the arrangement of the vessels and fibers and the thickness of the vessel walls, into groups corresponding in general to the then accepted taxonomic views. The black or red oaks, *Erythrobalanus*, were found to have the walls of the small summerwood vessels, and frequently also the walls of the large springwood vessels, six to eight microns thick, with round or elliptical lumina. The white oaks, *Leucobalanus* (*Lepidobalanus*), exhibited two anatomical types. The live oak type had thick-walled summerwood vessels, while the vessels of the other white oaks had thin walls only four microns thick and "not

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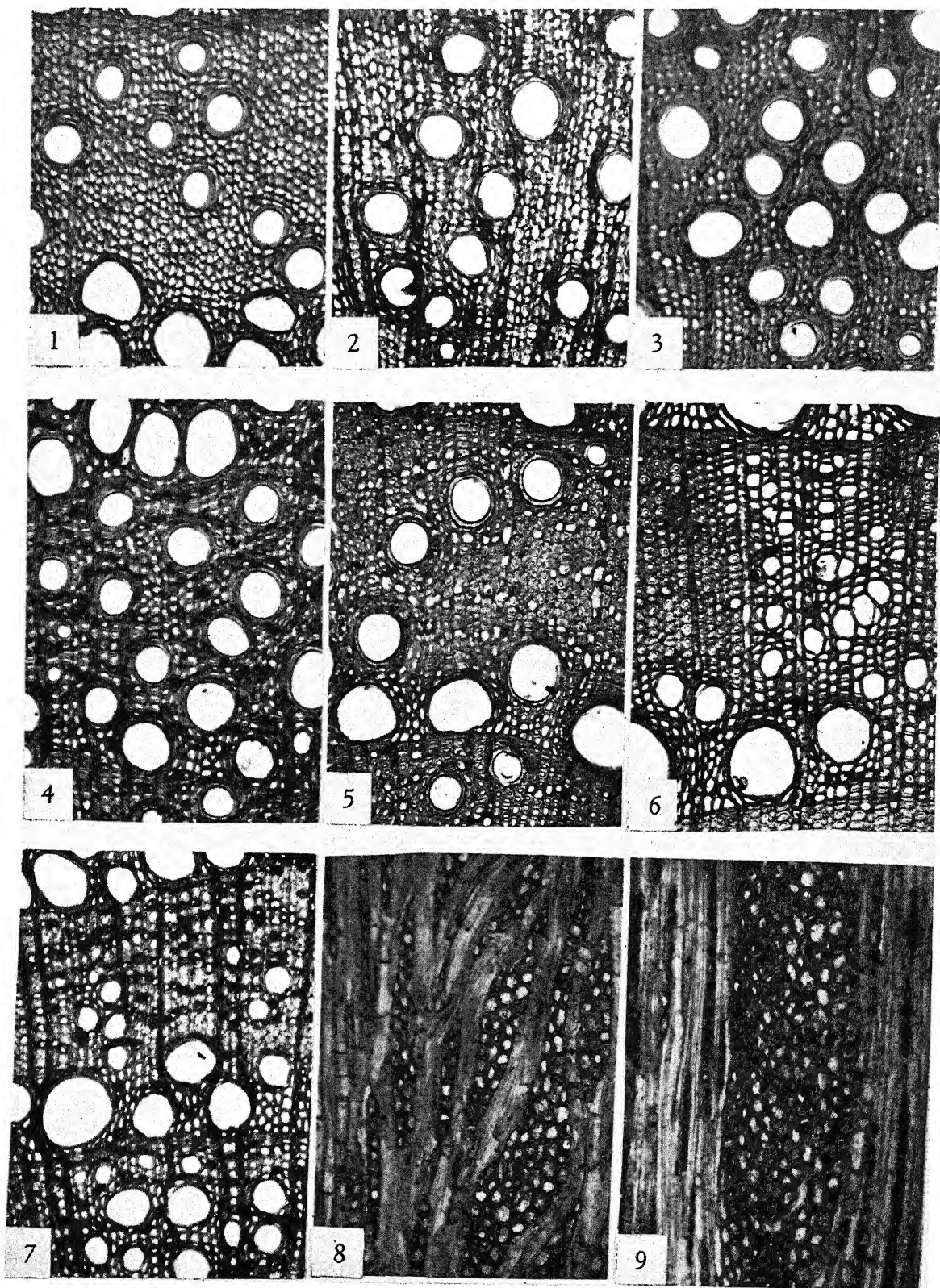


Fig. 1-9.—Fig. 1. *Quercus falcata*. Transverse section showing ring-porous wood with thick-walled, rounded summer-wood pores. $\times 180$.—Fig. 2. *Q. agrifolia*. Transverse section showing diffuse-porous wood with thick-walled, rounded

round." Only one exception was observed, *Q. Wislizeni*, a species in *Erythrobalanus* with thin-walled vessels.

Bailey (1910) examining forty-five species of American oaks, found that in *Lepidobalanus* the summerwood vessels are numerous, small, thin-walled, and angular, while in *Erythrobalanus* they are more scattered, larger, thick-walled, and circular. Tyloses are rare in the summer vessels of the red oaks, but frequent in the thin-walled vessels of the white oaks. In the live oaks, for which he suggested a new subgenus, *Biotobalanus*, the summerwood vessels are similar to those of the red oaks. They differ from the latter, however, in that the live oaks have aggregate rays, are diffuse-rather than ring-porous, and usually have no tyloses in the vessels. These so-called aggregate rays, at first considered to have arisen by a phylogenetic process of compounding, were shown by further investigation (Bailey & Sinoth, 1914; Barghoorn, 1940, 1941; Bailey and Howard, 1941) to have resulted from the disintegration or dissection of wide multiseriate rays.

Sudworth and Mell (1911) describe the woods of some thirty American oak species, classifying them according to the differences in the form and arrangement of the large and small vessels, wood fibers, and wood-parenchyma fibers. Although they do not make use of the thickness of the walls of the summerwood pores in their key, this character is mentioned in the descriptions of species or shown in the drawings.

Williams (1939) again made use of the difference in outline and thickness of the walls of the summerwood pores to delimit some fifty species of *Erythrobalanus* and *Leucobalanus*. Because of the contradictory nature of the accepted criteria, he proposed that the two groups be redefined.

"*Leucobalanus*: Summerwood pores angled, thin-walled [less than 3 microns]; leaves devoid of spinose teeth or bristle-tipped lobes."

"*Erythrobalanus*: Summerwood pores rounded, thick-walled [3 to 8 microns]; leaves wholly or in part with bristle-tipped lobes, apex apiculate, or margins with spiny teeth."

Eight species of white oaks were found to exhibit the "red oak" type of wood. Since he also considered their leaves to have bristle-tipped lobes or spiny teeth on the margins, he suggested the transfer from *Leucobalanus* to *Erythrobalanus* of the following eight species: *Q. Emoryi*, *Q. dumosa*, *Q. virginiana*, *Q. reticulata*, *Q. arizonica*, *Q. oblongifolia*, *Q. Engelmanni*, and *Q. Douglasii*.

In a more recent paper Williams (1942a) describes in detail the types of tyloses found in oak vessels and confirms the observation that tyloses are in

general more abundant in the white oaks than in the black oaks. In a third paper (1942b) he reemphasizes his earlier claim for the significance of late summerwood vessels as a means of distinguishing the subgenera of *Quercus*. Additional wood characters which are variously more or less loosely correlated with the vessel characters are discussed and the errors in Abromeit's pioneer effort are pointed out.

MATERIALS AND METHODS.—The stems used in this study were obtained mostly from herbarium specimens, although a few were obtained from trees growing in the vicinity of Washington, D. C. In table 1 is given a list of the 104 species examined, together with the name of the collector and collection number, or, if there was no number assigned, the date of collection.

All of the stems studied were at least three years old and from mature trees. In several species wood samples were taken from various parts of the same tree in order to observe the effect of age and exposure on the anatomy of the wood. Small blocks of stem were placed in the chuck of a sliding microtome and a jet of stem was played upon the block as the sections were being cut. Transverse and longitudinal sections were cut 22 microns thick and stained with safranin and fast green. Photographs were made, using XI and A filters, at a magnification of about 180 \times .

RESULTS.—Transverse sections of the stem show wide vascular rays radiating outward with much narrower uniseriate or biseriate rays between them. The wide multiseriate rays appear in tangential section as more or less homogeneous masses of round cells, as shown in *Q. grisea* (fig. 9). Occasionally the large rays appear to be broken up into aggregates of narrower rays by fibers and wood parenchyma which traverse them in a diagonally vertical direction, as in *Q. diversicolor* (fig. 8). These so-called aggregate rays are found both in *Lepidobalanus* (*Q. arizonica*, *Q. cordifolia*, *Q. diversicolor*) and in *Erythrobalanus* (*Q. durifolia*, *Q. palustris*). Intermediate stages, as evidenced by the frequent included fibers and wood parenchyma, are widespread in the genus.

The annual rings present a range from ring-porous to diffuse-porous. *Quercus falcata* (fig. 1), *Q. alba* (fig. 6), and *Q. Brayii* (fig. 7) illustrate the ring-porous type, with an abrupt decrease in size from the large springwood vessels to the much smaller summerwood vessels. The diffuse-porous condition appears in *Q. chrysolepis* (fig. 3) and *Q. grisea* (fig. 4), where large vessels are present throughout the year's growth. This variation appears related to a great extent to the climate and the length of the growing season. The deciduous oaks, such as

summerwood pores. $\times 180$.—Fig. 3. *Q. chrysolepis*. Transverse section showing diffuse-porous wood with thick-walled, rounded summerwood vessels. $\times 180$.—Fig. 4. *Q. grisea*. Transverse section showing diffuse-porous wood with thick-walled, rounded summerwood vessels. $\times 180$.—Fig. 5. *Q. arizonica*. Transverse section showing semi-ring-porous wood with thick-walled, rounded summerwood vessels. $\times 180$.—Fig. 6. *Q. alba*. Transverse section showing ring-porous wood with thin-walled, angular summerwood vessels. $\times 180$.—Fig. 7. *Q. Brayii*. Transverse section showing ring-porous wood with thin-walled, angular summerwood pores. $\times 180$.—Fig. 8. *Q. diversicolor*. Tangential section showing "aggregate" ray and uniseriate rays. $\times 180$.—Fig. 9. *Q. grisea*. Tangential section showing wide multiseriate ray and uniseriate rays. $\times 180$.

Q. stellata, *Q. alba*, *Q. falcata*, and *Q. coccinea*, are predominantly ring-porous, while the evergreen or partially evergreen oaks, as *Q. virginiana*, *Q. agrifolia*, *Q. grisea*, and *Q. arizonica*, tend to be semi-ring-porous to diffuse-porous.

In *Erythrobalanus*, although the springwood vessels of different species may have either thick walls or thin walls, the summerwood vessels are always

thick-walled, more or less scattered, and circular in outline, as shown in *Q. falcata* (fig. 1) and *Q. agrifolia* (fig. 2). Contrary to the statement of Abromeit (1884), *Q. Wislizeni* is no exception, but exhibits the typical red oak vessels.

In *Lepidobalanus* two types of summerwood pores occur. In thirty-one of the sixty species of white oaks examined, including *Q. grisea* (fig. 4) and *Q. ari-*

TABLE 1. *Types of summerwood vessels and leaf margins.*

Species, collector and number	Summerwood vessels			Leaf margins	
	Thin-walled, angular	Thick-walled, rounded	Round lobed	Mucronate	Aristate
LEPIDOBALANUS					
<i>Q. Alba</i> L. (Muller Oct. 10, 1936, Jan. 7, 1940; Tillson Dec. 21, 1940)....	×	..	×
<i>Q. albaefolia</i> C. H. Mull. (Muller 3535).....	×	..	×
<i>Q. arizonica</i> Sarg. (Muller 3584, 3712, 3743).....	..	×	..	×	..
<i>Q. basaseachicensis</i> C. H. Mull. (LeSeuer July 6, 1936).....	..	×	..	×	..
<i>Q. bicolor</i> Willd. (Muller June 8, 1938).....	×	..	×
<i>Q. boqueronae</i> Trel. (Matuda 1823).....	×	×	..
<i>Q. Brayi</i> Small. (C. H. & M. T. Mueller 651).....	×	..	×
<i>Q. breviloba</i> Sarg. (Muller 3140, 3141; Wynd & Mueller 308).....	×	×	..
<i>Q. carmenensis</i> C. H. Mull. (Wynd & Mueller 639).....	×	..	×
<i>Q. chihuahuensis</i> Trel. (Muller 3353, 3650).....	..	×	..	×	..
<i>Q. clivicola</i> Trel. & Muell. (Muller 2951).....	..	×	..	×	..
<i>Q. cordifolia</i> Trel. (Muller 2923).....	..	×	..	×	..
<i>Q. corrugata</i> Hook. (Standley 42834).....	..	×	..	×	..
<i>Q. depressipes</i> Trel. (Muller 3505A, 3580).....	..	×	..	×	..
<i>Q. derrumbaderoensis</i> C. H. Mull. (Mueller 2417).....	..	×	..	×	..
<i>Q. diversicolor</i> Trel. (Muller 3384, 3745).....	..	×	..	×	..
<i>Q. Douglasii</i> Hook. & Arn. (Kinsel 13).....	..	×	..	×	..
<i>Q. dumosa</i> Nutt. (Muller 2522; Kinsel 10, 36, 50; Clokey & Anderson 6572).....	..	×	..	×	..
<i>Q. durata</i> Jeps. (Belshaw 2238).....	..	×	..	×	..
<i>Q. Edwardsae</i> C. H. Mull. (Edwards 435).....	×	×	..
<i>Q. endemica</i> C. H. Mull. (Wynd & Mueller 540).....	..	×	..	×	..
<i>Q. Engelmanni</i> Greene (Muller 2505, 2523).....	..	×	..	×	..
<i>Q. filiformis</i> C. H. Mull. (Muller 3150).....	..	×	..	×	..
<i>Q. fusiformis</i> Small (Muller 2669).....	..	×	..	×	..
<i>Q. Gambelii</i> Nutt. (Wynd & Mueller 640; Ball & Solomon 5; Schneider 817).....	×	..	×
<i>Q. Garryana</i> Dougl. (Drexler 233).....	×	..	×
<i>Q. Greggii</i> Trel. (Muller 2926, 2928, 3239).....	..	×	..	×	..
<i>Q. grisea</i> Liebm. (Muller 3352A, 3714; Mueller Aug. 21, 1933).....	..	×	..	×	..
<i>Q. insignis</i> Liebm. (Schipp 1248).....	×	×	..
<i>Q. intricata</i> Trel. (Muller 3085, 3310).....	..	×	..	×	..
<i>Q. invaginata</i> Trel. (Muller 3136, 3161).....	..	×	..	×	..
<i>Q. LeSueuri</i> C. H. Mull. (LeSueur 1498).....	..	×	×
<i>Q. lobata</i> Née (Kinsel 46; Muller 2517).....	×	..	×
<i>Q. lyrata</i> Walt. (Muller June 8, 1938).....	×	..	×
<i>Q. macrocarpa</i> Michx. (Mueller Oct. 10, 1936).....	×	..	×
<i>Q. microlepis</i> Trel. & Muell. (Muller 2649, 2651).....	×	..	×
<i>Q. Mohriana</i> Buckl. (Wynd & Mueller 306).....	..	×	..	×	..
<i>Q. montereyensis</i> Trel. & Muell. (Muller 2981).....	×	×	..
<i>Q. Muhlenbergii</i> Engelm. (Muller 2582; Hermann 10363; Mueller Oct. 10, 1936).....	×	×	..
<i>Q. oblongifolia</i> Torr. (Muller 3586, 3666, 3738A, 3742).....	..	×	..	×	..
<i>Q. oleoides</i> Cham. & Schl. (Edwards 562).....	..	×	..	×	..
<i>Q. Pilarius</i> Trel. (Standley 71290).....	×	×	..
<i>Q. pilicaulis</i> Trel. (Trelease 38).....	×	×	..
<i>Q. polymorpha</i> Cham. & Schl. (Muller 2665).....	×	×	..
<i>Q. porphyrogenita</i> Trel. (Muller 2941, 3192).....	×	..	×
<i>Q. Pringlei</i> von Seemen (Muller 3206, 3222, 3238, 3300A).....	..	×	..	×	..
<i>Q. prinoides</i> Willd. (Hermann 10362).....	×	×	..

TABLE I. *Concluded.*

Species, collector and number	Summerwood vessels			Leaf margins	
	Thin-walled, angular	Thick-walled, rounded	Round lobed	Mucronate	Aristate
<i>Q. Prinus</i> L. (Muller 2550).....	×	..	×
<i>Q. pungens</i> Liebm. (Muller 3299).....	×	×	..
<i>Q. Sadleriana</i> R. Br. (Wheeler 3048).....	×	×	..
<i>Q. santaclarensis</i> C. H. Mull. (Shreve 7951).....	..	×	..	×	..
<i>Q. stellata</i> Wang. (Muller Jan. 7, 1940).....	×	..	×
<i>Q. supranitida</i> C. H. Mull. (Mueller 2169).....	..	×	..	×	..
<i>Q. Toumeyii</i> Sarg. (Muller 3601, 3748).....	..	×	..	×	..
<i>Q. trinidadensis</i> C. H. Mull. (Muller 2803).....	×	×	..
<i>Q. tuberculata</i> Liebm. (Muller 3611).....	×	..	×
<i>Q. turbinella</i> Greene (Clokey 7887, 7888).....	..	×	..	×	..
<i>Q. Vaseyana</i> Buckl. (Muller 3142).....	×	×	..
<i>Q. verde</i> C. H. Muell. (C. H. & M. T. Mueller 451; Muller 2942).....	..	×	..	×	..
<i>Q. virginiana</i> Mill. (Tidestrom 6933; Small & Carter Nov. 13-23, 1903)	×	..	×	..
PROTOBALANUS					
<i>Q. chrysolepis</i> Liebm. (Kinsel 40; Clokey 6995).....	..	×	×
<i>Q. tomentella</i> Engelm. (Kinsel 4).....	..	×	..	×	×
ERYTHROBALANUS					
<i>Q. affinis</i> Scheidw. (Muller 2423; C. H. & M. T. Muller 1232).....	..	×	×
<i>Q. agrifolia</i> Née (Muller 2504; Clokey 4895; Kinsel 49).....	..	×	×
<i>Q. albocincta</i> Trel. (Muller 3610).....	..	×	×
<i>Q. Canbyi</i> Trel. (C. H. & M. T. Mueller 1007; Schneider 1118; White 1545)	×	×
<i>Q. chrysophylla</i> Humb. & Bonpl. (Muller 2936).....	..	×	×
<i>Q. coccinea</i> Muench. (Muller 2586; Tillson Dec. 21, 1940).....	..	×	×
<i>Q. cupreata</i> Trel. & Muell. (Muller 2666).....	..	×	×
<i>Q. durifolia</i> von Seemen (Muller 3579, 3583).....	..	×	×
<i>Q. Emoryi</i> Torr. (Muller 3476, 3736A).....	..	×	×
<i>Q. Endlichiana</i> Trel. (Muller 2711b, 2950, 3588, 3698).....	..	×	×
<i>Q. epileuca</i> Trel. (Gentry 2218).....	..	×	×
<i>Q. falcata</i> Michx. (Muller Jan. 7, 1940).....	..	×	×
<i>Q. flocculenta</i> C. H. Mull. (Muller 2924).....	..	×	×
<i>Q. fulva</i> Liebm. (Muller 3509, 3563).....	..	×	×
<i>Q. graciliformis</i> C. H. Muell. (Mueller 565).....	..	×	×
<i>Q. Gravesii</i> Sudw. (Muller 3087, 3135, 3172).....	..	×	×
<i>Q. hypoleucoides</i> A. Camus (Muller 3713, 3741).....	..	×	×
<i>Q. hypoxantha</i> Trel. (Muller 3213, 3214, 3232).....	..	×	×
<i>Q. ilicifolia</i> Wang. (Martin & Erlanson 20).....	..	×	×
<i>Q. imbricaria</i> Michx. (Mueller Oct. 10, 1936).....	..	×	×
<i>Q. Kelloggii</i> Newb. (Kinsel 45).....	..	×	×
<i>Q. Knoblochii</i> C. H. Mull. (Muller 3600, 3607).....	..	×	×
<i>Q. laurifolia</i> Michx. (Muller Sept. 9, 1938).....	..	×	×
<i>Q. marylandica</i> Trel. (Muller 2580).....	..	×	×
<i>Q. maxima</i> Ashe (Martin & Erlanson 41).....	..	×	×
<i>Q. Morehus</i> Kell. (Johannson 3051).....	..	×	×
<i>Q. myrtifolia</i> Willd. (Pesoin, Oct., 1926).....	..	×	×
<i>Q. nigra</i> L. (Muller 2591).....	..	×	×
<i>Q. omissa</i> A. DC. (Muller 2918, 2921, 3508, 3560).....	..	×	×
<i>Q. pabillensis</i> C. H. Mull. (Taylor 141).....	..	×	×
<i>Q. palustris</i> L. (Muller Jan. 7, 1940).....	..	×	×
<i>Q. Phellos</i> L. (Muller Jan. 7, 1940; 2590).....	..	×	×
<i>Q. robusta</i> C. H. Muell. (Mueller 567).....	..	×	×
<i>Q. rysophylla</i> Weatherby (C. H. & M. T. Mueller 1123; Muller 2952)...	..	×	×
<i>Q. saltillensis</i> Trel. (Mueller 2419; Taylor 273).....	..	×	×
<i>Q. tardifolia</i> C. H. Muell. (Mueller July 3, 1932).....	..	×	×
<i>Q. tenuiloba</i> C. H. Muell. (Mueller 2122; C. H. & M. T. Mueller 1125)...	..	×	×
<i>Q. Tharpai</i> C. H. Mull. (Mueller Aug. 21, 1933).....	..	×	×
<i>Q. velutina</i> Lam. (Muller 2553; Mueller Oct. 10, 1936).....	..	×	×
<i>Q. vexans</i> Trel. (Muller 2804, 2824, 2943).....	..	×	×
<i>Q. viminea</i> Trel. (Muller 3587).....	..	×	×
<i>Q. Wislizeni</i> A. DC. (Muller 2513).....	..	×	×

zonica (fig. 5), the vessels are similar to those of the red oaks. The leaves of many of these species are thick and hard, and tend to be evergreen, while others are clearly deciduous. Mucronate tips, cusps, or "pseudo-bristles" are developed frequently in almost all of those white oaks having thick-walled, rounded summerwood pores. In the remaining twenty-nine white oak species the later-formed vessels are much smaller and more numerous, thin-walled and angular in outline, as illustrated by *Q. alba* (fig. 6) and *Q. Brayi* (fig. 7). In specimens taken from about the thirtieth annual ring of several red and white oak species the summerwood pores were larger than those in the much younger herbarium specimens, but the characteristic differences in shape and wall thickness noted above were still evident. On leaves of some of these species with thin-walled, angular summerwood pores, mucronate tips are entirely lacking, while in a few others there is a tendency toward the development of mucronate tips. Furthermore, the leaves of this group are distinctly deciduous. Tyloses, often abundant in these species, are also found in many other oak species, although not always in such large numbers. *Quercus chrysolepis* (fig. 3) and *Q. tomentella* of the intermediate subgenus *Protobalanus* have typical red oak summerwood vessels. In table 1 are listed the oak species examined, together with the type of wood and leaf margin found in each.

SUBGENERA AND SECTIONS OF AMERICAN QUERCUS.—The three subgenera recognized by Trelease (1924) are supported by the evidence of both wood anatomy and the conventional taxonomic characters. *Erythrobalanus* is set off from *Lepidobalanus* by its constantly tomentose inner surface of the acorn shell. This is fairly well correlated with the differences usually apparent in the bark, leaves, flowers, cup scales, and position of abortive ovules. Although any of these correlated characters may be lacking in one or another species, no instance is known of more than one character failing to appear in a single species.

The subgenus *Protobalanus* occupies a more or less intermediate position between the first two. It has the tomentose acorn shell and biennial fruition of *Erythrobalanus*, the short broad stigmas of *Lepidobalanus*, and characteristically lateral abortive ovules more or less like certain species of both groups.

Although the characters which set off the subgenera are, from species to species, not very constant, they are found in a great preponderance of the species. The study of wood anatomy was undertaken with the definite hope of finding contributing evidence by means of which to clarify the subgeneric lines, but no such evidence has been found. Williams' transfer of *Lepidobalanus* species with thick-walled, rounded summerwood vessels to *Erythrobalanus* is not supported by the examination of a larger number of species. The nature of the vessels in the late summerwood has been demonstrated not to distinguish the subgenera from one another but rather to separate one subgenus approximately into halves.

In *Lepidobalanus* about 50 per cent of the species studied have summerwood vessels which are thick-walled and rounded like those of *Erythrobalanus* and *Protobalanus*. These *Lepidobalanus* species characteristically have leaves with mucronately tipped teeth. The remainder of the subgenus is characterized by summerwood vessels which are thin-walled and angular in outline and by leaves with a tendency toward broadly rounded teeth or lobes (but many species more or less constantly develop mucronate tips). The round-lobed and thin-walled group belongs in the section *Robur* of Spach (1842), and the sectional name *Prinopsis* Schwarz (1936) has been applied to some of the mucronate-tipped and thick-walled species. It seems that there is no clear distinction between these two types.

The following diagnostic key presents the subgenera as they are now understood.

Acorn shell glabrous within, cup scales keeled or corky-thickened basally, abortive ovules basal or rarely lateral, styles short, stigmas broad and abruptly dilated, leaves, if toothed or lobed, with broadly rounded teeth or lobes or merely mucronate tips, not aristate-tipped by projection of the veins.

Subgen. *Lepidobalanus* Oersted

Cotyledons unequal, the radicles therefore lateral and obliquely directed (all tropical American species)

Sect. *Macrobalanus* (Oersted)²

Cotyledons equal, the radicles therefore apical and apically directed.

Sect. *Robur* Spach³

Acorn shell densely tomentose within, abortive ovules apical or lateral, very rarely basal.

Cup scales somewhat thickened basally and conspicuously tomentose, abortive ovules lateral, styles short, stigmas abruptly dilated, leaves, if toothed, with spinose or rarely aristate tips. Subgen. *Protobalanus* Trelease.

Cup scales flat or very rarely thickened basally, inconspicuously pubescent or glabrate, abortive ovules apical or rarely lateral or even basal, styles elongate and very gradually dilated to form the stigmas, leaves, if toothed, with aristate tips formed by the projection of the veins.

Subgen. *Erythrobalanus* (Spach) Oersted

SUMMARY

The wood anatomy of 104 species of American *Quercus* has been examined and the anatomical characters compared with the conventional taxonomic characters in an effort to establish a correlation between the two types of data. The well known character of red oak (*Erythrobalanus*) wood in which the summerwood vessels appear rounded in cross section and have thick walls (greater than three microns) was found to occur without exception in the red oaks. The white oaks (*Lepidobalanus*) have been regarded as characterized by summerwood vessels with thin walls (less than three microns) and angular in outline. Exceptions to this were considered by Williams as belonging to *Erythrobalanus*. About 50 per cent of the *Lepidobalanus* species examined in the pres-

² Subgenus *Macrobalanus* Oersted, treated as the genus *Macrobalanus* by Schwarz.

³ Includes section *Prinopsis* Schwarz.

ent study exhibited red oak wood characters. These characters are correlated very poorly with evergreen habit and the presence of mucronate tips on the leaves; they are not at all correlated with the conventional taxonomic characters of the subgenera. The anatomical characters of the wood, therefore, do not constitute a basis for subgeneric division and

only roughly indicate a possible division of the white oak subgenus into sections.

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THE DISTRIBUTION OF AUXINS IN BULBS OF LILIUM LONGIFLORUM¹

William S. Stewart and Neil W. Stuart

WHILE AUXINS are known to occur in seeds and certain other storage organs (Went and Thimann, 1937), no previous tests of lily bulbs for these substances appear to have been made. Easter lily (*Lilium longiflorum* Thunb.) bulbs are always subjected to a period of cool storage at about 35°F. prior to forcing for flower production. This practice is followed primarily to maintain the bulbs in good condition for successive plantings throughout the year, but it is also known to exert a marked effect in accelerating flowering. Under the most favorable conditions flowers are produced from cool-stored bulbs in about one-third the time required for flower production from bulbs not cool-stored. This acceleration in flowering is accompanied by longer internodes and fewer leaves, suggesting that changes in the growth-regulating substances may be involved. Before undertaking a study of the possible role of auxins in accelerating flowering of cool-stored bulbs of Easter lilies, these preliminary studies were made: first, on the distribution of auxins in various parts of

the bulb; and second, on the changes in this distribution associated with the initiation of growth.

MATERIALS AND METHODS.—The Easter lily bulbs used in the experiments reported here were of the Creole variety grown in Louisiana in 1940, and forced in the greenhouses at the United States Horticultural Station, Beltsville, Maryland, during the winter and spring of 1940-1941. The forced bulbs were allowed to remain in the greenhouses until fully ripened. Late in July the bulbs were harvested, graded, packed in moist peat moss and stored in a cellar where the temperature ranged from 70° to 85°F. On November 13 duplicate samples of the dormant bulbs 12 to 14 cm. in circumference were dissected and their relative auxin content determined. Each bulb was dissected into five portions (fig. 1) as follows: (1) the outer scales removed until the remaining portion of the bulb was 8 cm. in circumference; (2) the inner scales, or remaining scales down to the growing point; (3) the basal plate of the bulb on which the scales are borne; (4) the stem which for part of its length is surrounded by the basal plate;

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and (5) the stem tip, or the apical 3 mm. of the stem. Upon dissection, the respective parts from fifteen bulbs were combined, weighed, and put directly into ether. The outer scales were extracted with 450 cc. of ether, the inner scales with 300 cc., the basal plate 150 cc., the stem 50 cc., and the growing point 50 cc. In the stem tip and stem the ratio of tissue surface to

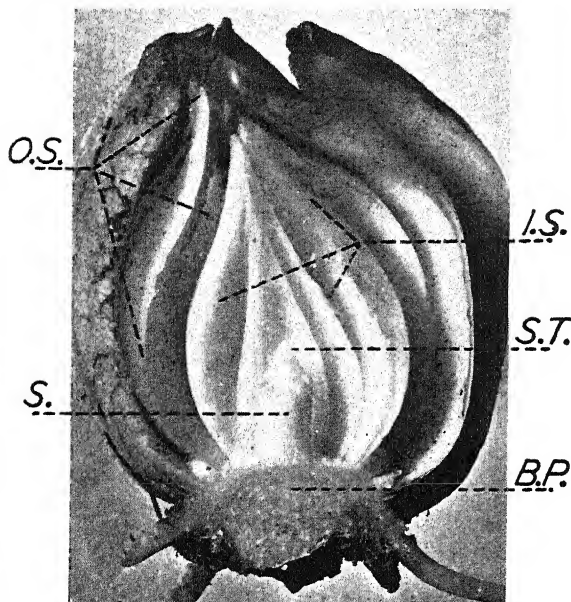


Fig. 1. Longitudinal section of a bulb of *Lilium longiflorum* showing regions sampled separately for study of auxin content. S.T., stem tip; S., stem; B.P., basal plate; I.S., inner scales; O.S., outer scales.

tissue volume is greater than in the other parts of the bulb. The effect of these differences on auxin extraction is not known.

Although auxin extraction methods are not yet perfected, it is generally agreed that by the use of an arbitrary but uniform extraction method, amounts of auxin in various tissues may be compared. However, absolute amounts cannot be determined, for as yet exhaustive extraction methods for auxin are not known for every plant tissue. In these experiments peroxide-free ethyl ether was used to extract the auxin (van Overbeek, 1938a). The whole tissues were extracted overnight in this solvent. The ether was then decanted and evaporated to dryness in a shell vial. The residue containing auxin was taken up in a small volume of 1.5 per cent melted agar and molded into blocks for auxin determination by the standard *Avena* test. Any waxes or other solids deposited in the vial on evaporation of the ether were melted and shaken into the agar by placing the vial in a rapidly boiling water bath for a very short time. Continuous records of *Avena* coleoptile curvature with time, even when the curvatures were caused by extracts of the highest auxin concentration, showed no "cross transport" or inhibition due to high concen-

tration. As the amounts of extracted auxin were considerably less than those needed to cause a maximum angle of curvature of *Avena* coleoptiles, auxin determinations of dilutions of the original extract were not made.

The first experiments showed that only the growing point and stem yielded sufficient amounts of auxin to cause definite *Avena* coleoptile curvatures. The kymograph technique was used to measure the smaller amounts of auxin in the other tissues (Schneider and Went, 1938). By inserting a 0.12 mm. diameter silver wire into the primary leaf, curvatures of the coleoptile of less than one degree become magnified and are readily and accurately measurable, since the tip of the coleoptile is in effect extended about 6 cm. Furthermore, interference of regular auxin curvatures by growth inhibitors extracted along with the auxin are made apparent by automatic recording of the changing curvatures of the coleoptile every four minutes over a period of 280 minutes rather than just at the end of 90 minutes as in the standard *Avena*

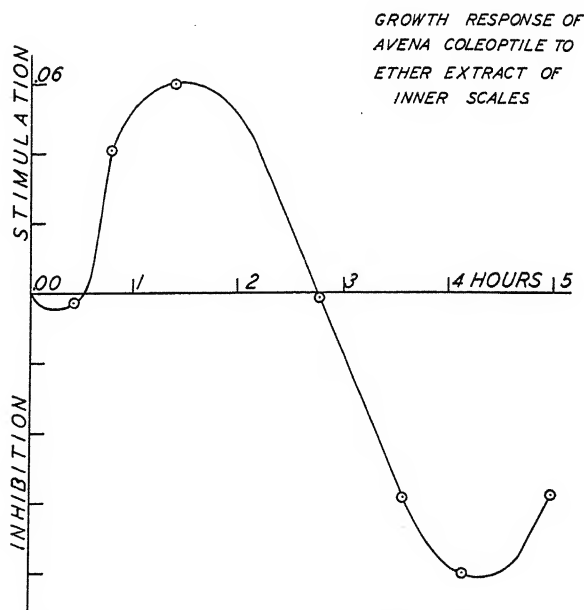


Fig. 2. Response of *Avena* coleoptiles with time, to growth regulators extracted from the inner scales of *Lilium longiflorum* on Nov. 17. Ordinates indicate micrograms of indoleacetic acid equivalents per kilogram fresh weight. Above the horizontal axis, the positive values indicate growth stimulation or auxin predominance; below this axis, the negative values indicate growth inhibition. Abscissas indicate time in hours. Each determined point represents the average of twelve coleoptiles.

test. Although such an inhibiting substance was extracted from the outer and inner scales and from the basal plate, this substance did not manifest itself at the 90-minute interval when the auxin curvatures were measured (fig. 2). After this time it caused a growth inhibition of the coleoptile, often resulting in strong "positive" curvatures, and in a marked plas-

TABLE 1. *Auxin distribution in bulbs of Lilium longiflorum on November 13.*

Tissue extracted	Fresh weight of tissue (grams)	Volume of agar (cc.)	<i>Avena</i> coleoptile curvature from extract (degrees)	<i>Avena</i> coleoptile curvature from indoleacetic acid (50 μ g./l.)(degrees)	Indoleacetic acid equiv./kg. fresh weight (micrograms)
Outer scales A ^a	183.6	0.3	4.5	12.3	0.029
Outer scales B	177.4	0.3	6.1	12.3	0.041
Inner scales A	114.3	0.5	6.8	14.7	0.093
Inner scales B	121.9	0.5	5.1	14.7	0.070
Basal plate A	27.1	0.3	2.6	12.3	0.115
Basal plate B	25.1	0.3	1.5	12.3	0.072
Stem A	0.976	0.3	13.3	14.3	14.3
Stem B	1.056	0.3	16.1	14.3	16.0
Stem tip A	0.277	0.3	19.1	14.3	72.5
Stem tip B	0.214	0.3	17.1	14.3	83.9

^a A and B indicate separate samples.

molysis and collapse of the coleoptile cells under the agar block. Apparently the substance caused the inhibition by killing the coleoptile cells. In this respect it is quite different from a substance obtained from radish (*Raphanus sativus* L.) which causes coleoptile growth inhibition in less than 90 minutes without the plasmolysis and collapse of the coleoptile cells (Stewart, 1939). Units of auxin in the various parts of the bulb are expressed arbitrarily as micrograms of indoleacetic acid equivalents per kilogram of fresh weight of tissue.

RESULTS AND DISCUSSION.—Extractions made on November 13 showed that the stem tip contained about one thousand times the concentration of auxin present in the scales and basal plate. The stem contained one-fifth the amount of auxin in the stem tip and two hundred times the concentration present in the scales and basal plate (table 1). The full significance of the high concentration of auxin in the meristems is of course unknown. Indoleacetic acid at a concentration of 100 milligrams per liter when applied to seedling bean stems induces a mobilization of food material toward the place of application (Mitchell and Stuart, 1939). It seems possible then that the relatively high concentration of auxin in the stem tip may have a similar function and mobilize food material toward the growing region.

The differences in auxin content of the various portions of the dormant lily bulbs made it desirable to investigate how this distribution might change as the bulbs resume active growth. Accordingly, 200 bulbs were packed in moist peat moss on November 13 and stored at a constant temperature of 77°F. These bulbs were from the same lot used for the determinations just described. From previous experience (Brierley, 1941) it was known that bulbs maintained at summer temperatures for some months would start active growth as soon as they were provided with moisture.

At five intervals between November 13 and December 15, duplicate random samples of fifteen bulbs each were withdrawn from storage, dissected, and their auxin concentration determined. The results are summarized graphically in figures 3 and 4. Auxin

concentration in the stem tip on November 6 is also given; the auxin concentration in the other tissues on this date were not obtained. The data show that during the storage there was a continual decrease in

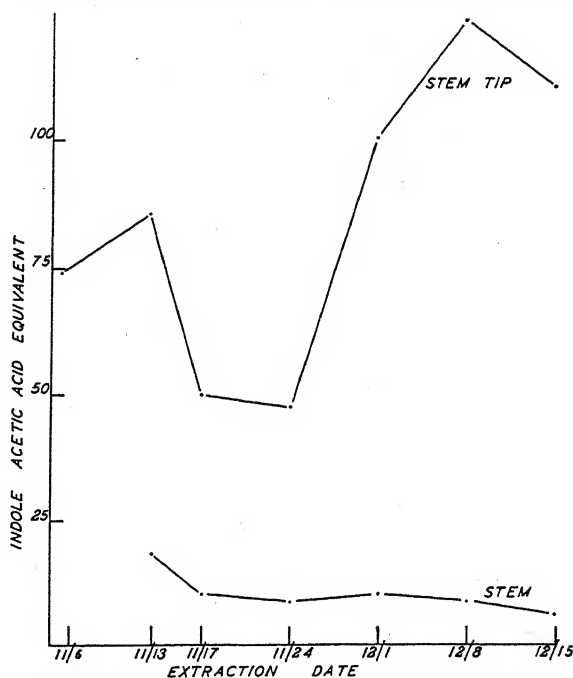


Fig. 3. Auxin, as micrograms of indoleacetic acid equivalents per kilogram fresh weight, extracted from stem tip and stem of bulbs of *Lilium longiflorum*. Bulbs kept at 77°F.

auxin in the outer and inner scales and in the stem. In the basal plate and growing point there was an initial decrease followed by a definite increase (figs. 3 and 4). In the growing point the auxin content had increased about a third. The significance of these changes is again a matter for conjecture. It has been reported that actively elongating stem tissues exhibit a lower auxin concentration than do their apical meristems (van Overbeek, 1938b). This relationship

appears to exist in lily bulbs. Simultaneously with the beginning of accelerated growth, the ratio of auxin in the apical meristem to that in the stem increased about fourfold. This was due to increase of auxin in the growing point as well as to its decrease in the stem. On December 15 the concentration of

(2) stem axis, 5.20; (3) basal plate, 0.08; (4) inner scales, 0.03; (5) outer scales, 0.02.

During the storage period the bulbs produced new roots, and the stem elongated about two centimeters. It is noteworthy that the increase in auxin occurred in the regions of meristematic activity, *i.e.*, in the basal plate where there are numerous root primordia and in the growing point. The auxin relations in the lily bulb are somewhat comparable with those in dormant vegetative buds where an increase in auxin has been found to precede the initiation of accelerated growth (van Overbeek, 1938b). In bulbs the increased auxin in the basal plate and in the apical meristem might stimulate the formation of new roots and promote accelerated stem growth. Studies of auxin distribution in freshly harvested, cool-stored bulbs are now in progress.

SUMMARY

Large differences in relative amounts of auxin were found in different parts of Easter lily bulbs on November 13, four and a half months after harvest and after storage in moist peat moss at 70° to 85°F. The apical 3 mm. of the stem axis contained about one thousand times the concentration of auxin present in the scales and basal plate. The remainder of the stem contained one-fifth the relative amount of auxin in the apical meristem. During subsequent storage from November 13 to December 15 at 77°F. and with sufficient moisture for active growth, there was a continual decrease of auxin in the scales and in the stem. In the basal plate and stem tip there was an initial decrease followed by a definite increase of auxin. The possible relationships between auxin changes and growth responses of the bulbs are discussed.

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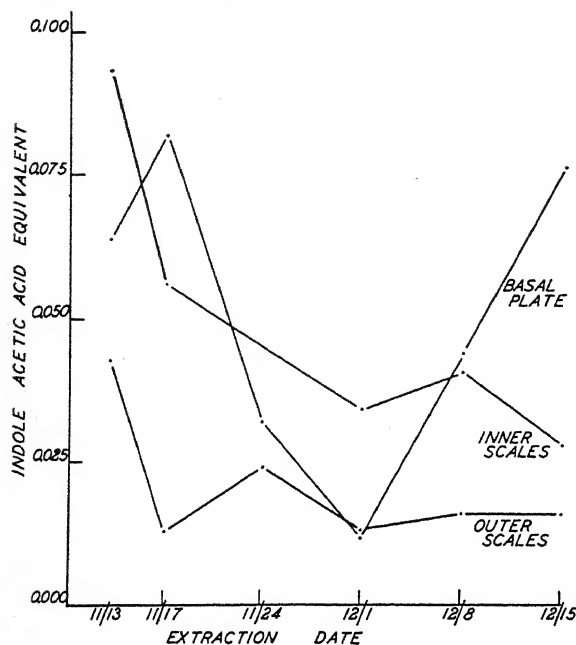


Fig. 4. Auxin, as micrograms of indoleacetic acid equivalents per kilogram fresh weight, extracted from basal plate, inner scales, and outer scales of bulbs of *Lilium longiflorum* from Nov. 13 to Dec. 15. Bulbs kept at 77°F.

auxin in the stem tissue was only about one-twentieth that found in the growing point. At this time the relative auxin concentrations of the several regions of the bulb were: (1) apical 3 mm. of stem axis, 110.5;

CELL GROWTH AND DIVISION IN LIVING ROOT MERISTEMS ¹

Robert T. Brumfield

KNOWLEDGE CONCERNING the processes of growth and division of individual meristematic cells as they develop to maturity is clearly of importance for an understanding of the factors involved in cell differentiation and plant morphogenesis. Little is known about these processes in multicellular tissues except from fixed and sectioned material, the limitations of which are obvious. The most significant evidence can be obtained only by the use of living tissue wherein the same cell can be observed throughout its development. Recently a technique has been devised whereby growth and division of single cells in living root meristems can be studied. Results obtained by the use of this method are reported in this paper.

Sinnott (1939) has pointed out that the epidermal cells in root meristems of many small-seeded grasses can be clearly seen in the living condition since the root cap consists of but a few loose cells at the extreme tip. Seedlings are grown on microscopic slides covered with moist lens paper and kept in covered Coplin staining jars with a little water at the bottom of the jars. The growing seedling is flooded with water, covered with a supported cover glass, and placed under the microscope for observation of the growing root. By means of camera lucida drawings made at successive intervals of time, it is possible to observe cell division and growth.

Using this technique, Sinnott and Bloch (1939a) made a study of root hair formation in *Phleum pratense*, *Poa trivialis*, *Sporobolus cryptandrus*, and *Chloris gayana*. It was found that in *Phleum* and *Poa* the last cell division is an unequal one which results in a small trichoblast or root hair-forming cell and a larger cell which does not form a root hair. The trichoblast is always toward the tip of the root and the hairless cell toward the base. During elongation, the trichoblasts grow less than the hairless cells. In *Sporobolus* and *Chloris* the divisions were found to be more nearly equal and both daughter cells may form root hairs.

In a later paper (Sinnott and Bloch, 1939b), especial attention was given to "sliding growth" and differential wall growth, using *Phleum* and *Sporobolus* for observation. All the evidence obtained indicated that sliding growth does not occur. Differential wall growth was apparent in that: (1) parts of the walls of hairless cells opposite trichoblasts grow less than parts opposite other hairless cells; (2) under certain conditions, cells grow more in the middle portions than near the ends; (3) the end of a cell toward the base of the root attains its final size sooner than the apical end of the same cell.

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These observations show that the differences in size of large cells and small ones are a result not only of unequal divisions but also of differences in their growth rates. The present investigation was undertaken to determine the growth rates of single cells and their descendants as they pass from the meristematic condition to maturity, thus obtaining more

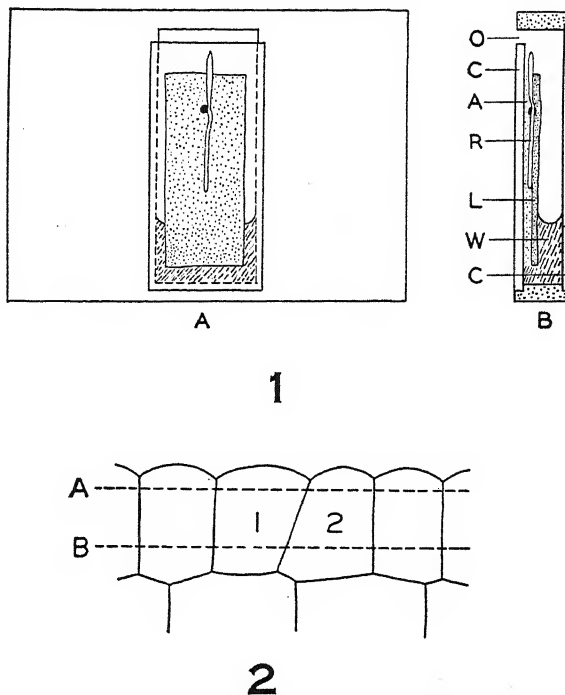
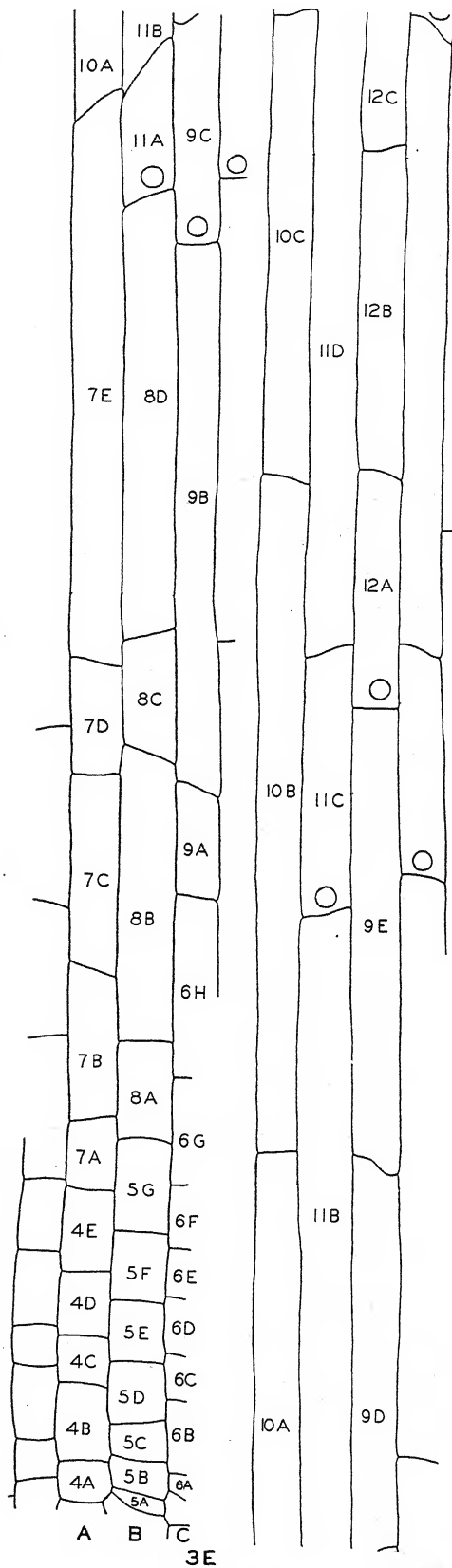
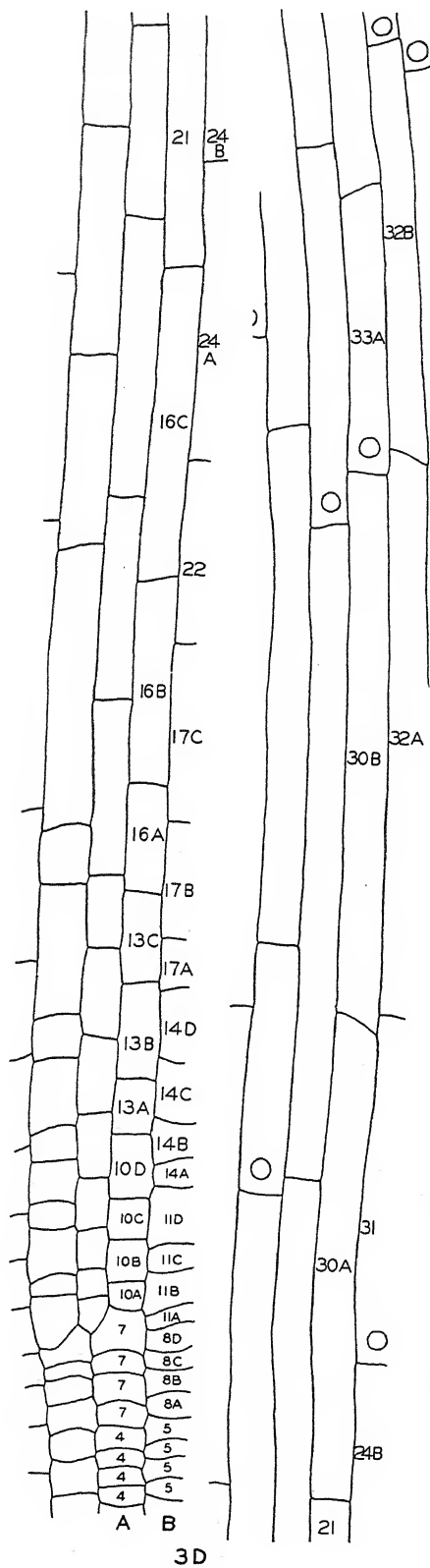
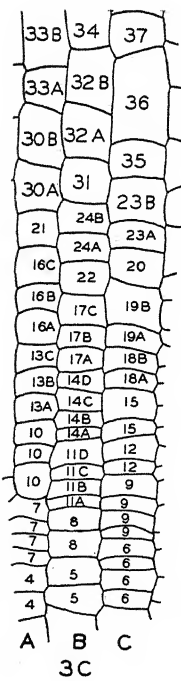
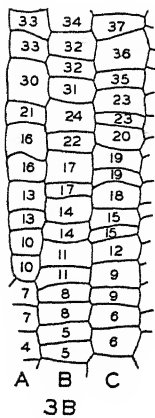
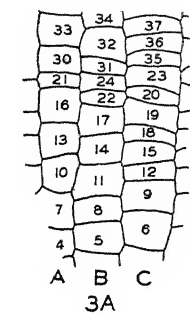


Fig. 1-2. Showing method used to photograph growing roots of grass seedlings.—Fig. 1. Diagram of moist chamber. A, front view. B, section through the chamber. O, opening for gaseous interchange; C, cover slip; A, agar; R, root; L, lens paper; W, water.—Fig. 2. Diagram showing effect of different levels of focus and an oblique wall on apparent cell length in photomicrographs of living roots. At focus A cell 1 would appear longer, and cell 2 shorter, than at focus B.

precise data on the differences in growth of hairless cells and trichoblasts.

MATERIALS AND METHODS.—*Phleum pratense* was chosen for the experiment, because in this species trichoblasts and hairless cells are clearly differentiated. Seed was obtained from the United States Department of Agriculture, Bureau of Plant Industry, Division of Forage Crops and Diseases, Washington, D. C., through the courtesy of M. A. Hein. The seed was from the 1940 harvest and was of a strain designated as F. C. no. 22,608. The seeds were evidently heterogeneous genetically, having a wide range in germination time and giving about 3 per cent albino plants.



There were several difficulties in the method of observation used by Sinnott and Bloch (1939a) which have been avoided in the present investigation by the use of a new technique. Their procedure requires that the root be put in a horizontal position for the period of observation. After a few minutes on the horizontal stage, the root twists somewhat, probably as a result of alteration in its position with respect to gravity, and does not grow normally (*cf.* Sinnott, 1939). Thus cells on the upper surface of the root at one observation period may, as a result of twisting, be turned to one side of the root at the next period. This behavior often makes it impossible to find the same group of cells at successive intervals of time. Moreover, it is difficult to make camera lucida drawings fast enough to obtain satisfactory growth curves for a group of rapidly growing cells, since the elongation of the root constantly changes the image of the cells in relation to the drawing. These difficulties were avoided in the present investigation by growing the seedling in such a way that it could be observed without changing its position, and photomicrographs were made rather than camera lucida drawings.

A chamber was constructed by cutting an opening about 20 mm. x 46 mm. in a piece of cardboard about 50 mm. x 77 mm., and 2 mm. thick, with the long axis of the opening at right angles to the long axis of the cardboard. One side of the opening was covered with a microscopic cover glass 24 mm. x 50 mm. and sealed with paraffin. A cover slip 24 mm. x 45 mm. was placed over the other side. Since the second cover slip was shorter than the opening in the cardboard, a space was left uncovered for gaseous interchange. Before the second cover was put in place, it was smeared with agar, a germinating seed placed near one end, and the agar and seedling covered with a strip of moist lens paper. The cover slip was put in place and sealed with paraffin, the seedling being inside the chamber thus formed. A few drops of water were introduced, and the lens paper acted as a wick keeping the seedling and agar moist (fig. 1). The cardboard was placed on the stage of a horizontal microscope with the long axis of the chamber in a vertical position, the seedling being at the upper end of the chamber and the cover glass bearing the seedling being nearest the objective. In this way the growing root was kept in the same position at all times, and photomicrographs could be taken without disturbing the growing seedling. The agar was of 0.25 per cent concentration and contained 0.25 gm. of NH_4NO_3 , 0.1 gm. of KH_2PO_4 , 0.1 gm. MgSO_4 and 0.05 gm. CaCl_2 per liter. The experiment was carried out at room temperature, about 25°C.

Photomicrographs were taken with a Bausch and Lomb type "H" camera in the horizontal position. A Bausch and Lomb mercury arc lamp with a water-

cooling cell and a Wratten filter number B-58 (green) served as the light source. The root was illuminated with this lamp only when photographs were being taken. At other times it was illuminated by overhead lamps of the usual intensity for laboratory rooms.

The seedling was allowed to grow for several hours to become adjusted to its new position, and then photographs were taken at half-hour intervals for a period of thirty and one-half hours, starting at 10:30 p.m., June 20, 1941. A 40X fluorite oil immersion objective and a 7.5 X eyepiece were used for the small cells close to the root apex. It was necessary, of course, that both the cover slip bearing the seedling and the agar layer be thin; otherwise the root would have been held beyond the focus of the objective. The thinness of the moist chamber made it possible to focus the condenser, giving better resolution in the photographs. A 10 X objective and a 15 X eyepiece were used to photograph the longer cells farther behind the root apex. Mineral oil was used as the immersion medium, and it was found unnecessary to remove the oil from the cover when changing from the immersion objective to the low power one. Negatives were taken on Super XX Panchromatic cut film $3\frac{1}{4}'' \times 4\frac{1}{4}''$ and enlarged in printing to give a magnification of 757 times for the small cells near the tip and of 412 times for the longer cells. Thus a successive series of photographs was obtained from which cell length could be easily measured. Measurements were made by placing the points of a small pair of dividers on each end of the cell at the juncture of its transverse and longitudinal walls and then transferring to a millimeter rule, readings being made to tenths of a millimeter. The data were later corrected to microns. The growing root was measured at each half-hour interval by removing the camera bellows and substituting an eyepiece fitted with an ocular micrometer for the one used in taking the photographs.

Although the root was not subjected to changes in position with respect to gravity, there was a tendency for the tip to rotate around the long axis of the root. This rotation carried cells photographed at an earlier period out of the microscope field on one side and brought cells not previously photographed into the field on the other side at a later period (compare figures 3C and 3D). This limited the number of cells which could be followed throughout development. Also, some inaccuracy in measurement of cell length resulted from slight differences in the level of focus at which successive photographs were taken, since the end walls of the epidermal cells are not always at right angles to the long axis of the root but may be somewhat oblique. The effect that an oblique end wall and different levels of focus may have on the apparent length of a cell in the photographs is shown

Fig. 3, A-E. Drawings of a group of epidermal cells and their descendants at successive intervals of time. Cell 5 is nearest the root apex and about 0.07 mm. behind it in A. A, June 20, 10:30 P.M. B, June 21, 4:30 A.M. C, June 21, 10:30 A.M. D, June 21, 5:30 P.M. E, June 22, 4:00 P.M. At the completion of division in each cell, the individuals of the resulting complex were designated by a letter, cells nearest the root apex being given the letter A, the next B, etc. Traced from photomicrographs with the aid of a pantograph X 284.

diagrammatically in figure 2. At focus *A* cell 1 would appear longer than at focus *B*; similarly, cell 2 would appear longer at focus *B* than at *A*. The percentage of error thus introduced is small, however, and decreases as the cell increases in length.

RESULTS.—The results are best presented by a series of drawings and growth curves. Figures 3A-E show the same group of meristematic cells at successive intervals during their development. In each case the apical end of the root is towards the bottom of the plate, and at the beginning of the experiment the group of cells was about 0.07 mm. behind the root tip (root cap not included). After the completion of division in each cell the individuals of the resultant complex were designed by a letter, as for example 11A, 11B, 11C, and 11D (fig. 3C), cells nearest the root apex being given the letter A, the next B, etc.

It is evident from the figures that cells near the tip undergo more division than those farther behind it. Thus cells 33 and 32 divided only once, each forming two daughter cells. Cells 10 and 11 divided twice, each giving rise to four daughter cells. Cell 5 divided three times, resulting in a complex of seven cells (the basal cell did not divide at the time of the third division). Cells farther from the tip than 33 and 32 did not divide.

The growth curve for cell 11 and its resultant complex of cells is shown in figure 4. The logarithm of cell length is plotted against time. At the beginning of the experiment, cell 11 was single (fig. 3A). Between 12:00 A.M. and 12:30 A.M., June 21, it divided (fig. 3B). Between 7:00 A.M. and 7:30 A.M., June 21, the more basal of the two daughter cells divided; between 8:30 A.M. and 9:00 A.M., June 21, the more apical of the two daughter cells formed at the first division divided, giving the complex four cells (fig. 3C). No further divisions occurred, and the complex reached its final size consisting of four cells. Two of them (11A and 11C in figure 3C) were trichoblasts and two (11B and 11D, figure 3C) were hairless cells. Measurements were made of one longitudinal wall (the wall between cell row A and cell row B in figures 3A-E) of the whole complex regardless of the number of cells into which it divided. The times at which divisions occurred are indicated on the curve in figure 4 by arrows. The gaps in the curve are due either to the cell being out of focus, making measurement impossible, or to the rotation of the root which carried the cell out of the microscope field for a short period. The wavy character of the lower part of the curve is probably due to different levels of focus and an oblique end wall rather than to rhythms of elongation. Variation in the level of focus tends to occur at more or less regular intervals, since the tip of the growing root moves downward in a spiral fashion. This movement results in the tip being close to the cover slip at some periods and farther away at others, thus causing periodic variation in the level of focus.

As seen from figure 4, the cell and its resultant complex passed through two growth phases, elongation proceeding at a constant exponential rate in

both. These two phases are represented by the linear parts of the curve. In the first phase (the lower part of the curve) the rate was about 5.4 per cent per hour,² this rate remaining constant for about fourteen hours. At the end of this phase, the rate began to increase and continued to do so until a second constant rate was established, this rate being about 41.2 per cent per hour. The period of increasing rate lasted for about nine and one-half hours and apparently represents a transition from the first growth phase to the second. In the second phase, the growth rate remained constant at 41.2 per cent per hour. This phase was of short duration, lasting for about four hours only. At the end of the second phase the growth rate fell off to zero quickly.

Cell division occurred only during the first growth phase. At the end of the first phase the vacuoles increased in size, doing so earlier in the long hairless cells than in the shorter trichoblasts. Root hairs began to appear as soon as the complex reached its final length.

Growth curves for cell complexes 5, 8, 10, 11, 16, and "31-32" are shown in figure 5. It was desired to compare the growth of complexes in all parts of the meristematic region. In the basal part of the meristem, however, trichoblasts and hairless cells had already been differentiated. Growth in either of these two types of cells alone would probably not be comparable to a complex consisting of both types. For this reason cells 31 and 32 were measured together as a single complex, cell 31 being a trichoblast and 32 a hairless cell (fig. 3A). The longitudinal wall measured in each complex was that one between cell row A and cell row B (fig. 3A). The walls measured in cells 5 and 8 were the same length at the beginning of the experiment, and the two curves coincide in the parts representing the first phase of growth. The growth rate of cell 8 increased sooner than that of cell 5, however. Neither of them completed its growth during the period of observation.

As seen from figure 5, the growth curves for all complexes are quite similar, differing only in the length of that part of the curve which represents the first phase of growth. This length evidently depends on the position of the cell or complex with respect to the root apex at the beginning of the experiment, the first phase being longer for those cells near the root tip. This suggests that the first phase of growth occurs in a definite region or zone and each complex begins the transition to the second phase when it is carried out of this zone by the elongation of cells apical to it. Since the transition period and the second phase are similar for all complexes, these processes must also occur in definite zones. Thus the transition occurs in a second zone lying immediately behind the first zone and the second growth phase takes place in a third zone which lies behind the second. Elongation ceases at the end of the third zone.

² Calculated from the formula $A = Pe^{rt}$, in which *A* is the size after a given time, *P* the size at the beginning, *e* the base of the natural logarithms, *r* the rate, and *t* the number of time intervals.

TABLE 1. Showing the distance behind the root apex at which different complexes completed the first growth phase, the distance at which they began the second growth phase, and the distance at which elongation ceased.

Complex number	First growth phase completed	Second growth phase began	Elongation ceased
"31-32"	.14 mm.	.32 mm.	.97 mm.
16	.15 mm.	.33 mm.	1.01 mm.
10	.14 mm.	.31 mm.	.95 mm.
11	.15 mm.	.31 mm.	.95 mm.
8	.13 mm.	.30 mm.	—
5	.13 mm.	—	—
Average	.14 mm.	.31 mm.	.97 mm.

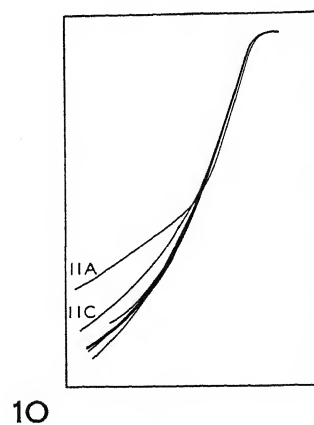
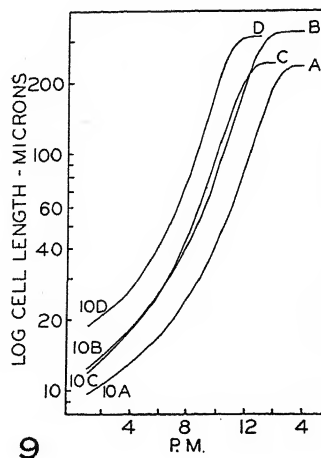
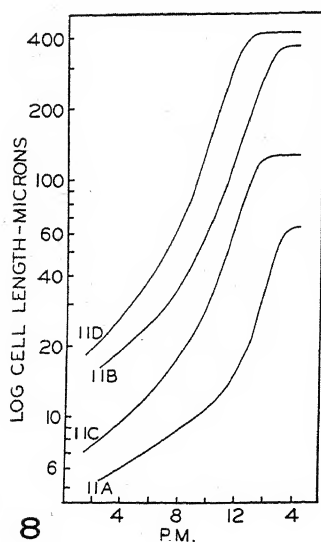
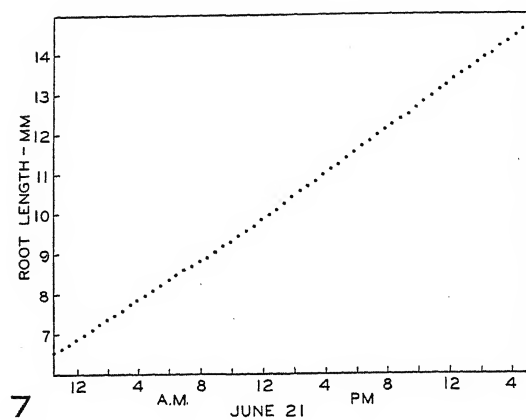
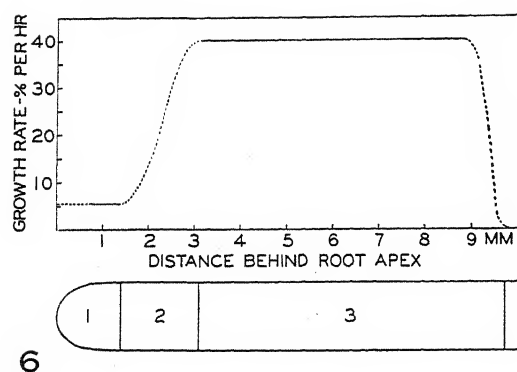
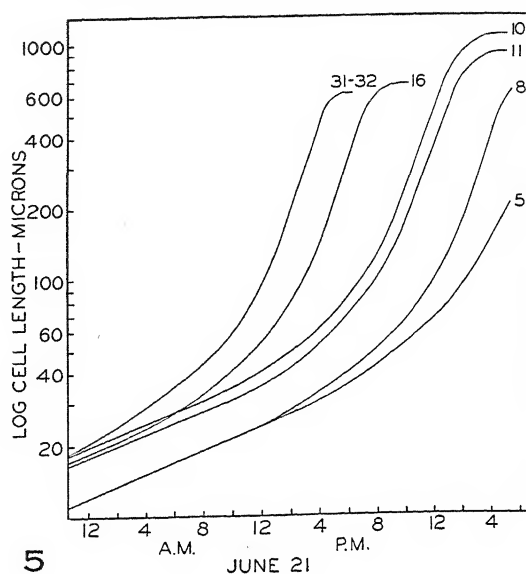
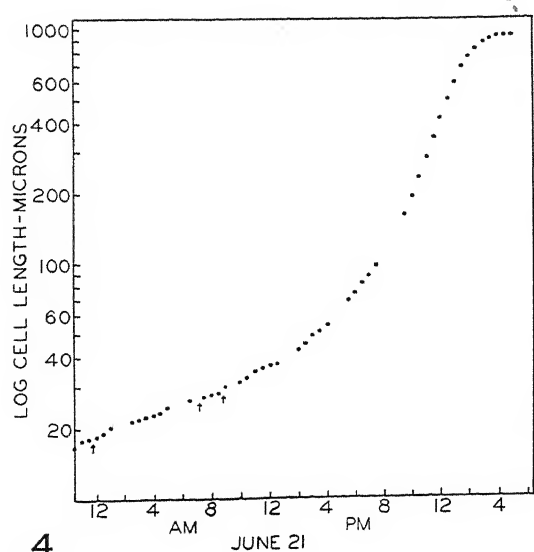
The length of each of these zones was determined by reference to the growth curves shown in figure 5 and by measurements made from the photographic record. The time at which a given complex passed from one zone to another was marked by a change in its growth rate, and the distance from the apical end of the complex to the tip of the root was measured from photographs taken at the time the change in rate occurred. Complex 10 passed into the second growth zone at about 11:00 A.M., June 21, as evidenced by an increase in its growth rate (fig. 5). The apical end of the complex was about 0.14 mm. behind the root apex (root cap not included) at that time, as measured from the photographic record. Thus complex 10 passed from the first zone to the second when it was about 0.14 mm. behind the root apex. Obviously, the basal end of any complex enters the second zone first. On doing so, the growth rate of the whole complex apparently increases, although the apical end may be still growing at the rate in zone 1. At the time the growth rate of the whole complex first shows an increase, the border line between the first and second zones lies somewhere between the two ends of the complex rather than at its apical end, and measuring to the apical end of the complex makes zone 1 appear somewhat shorter than it actually is. This does not introduce an appreciable error, however, since the complex is rather short at this time. At about 8:30 P.M., June 21, the whole of complex 10 had entered the third growth zone as indicated by the growth rate becoming constant (fig. 5). The apical end of the complex was about 0.31 mm. behind the root apex at that time, showing that the border line between zones 2 and 3 was about 0.31 mm. behind the root apex. The complex stopped elongation at about 4:00 A.M., June 22, indicating that the whole complex had just passed out of zone 3. At that time, the apical end of the complex was about 0.95 mm. behind the root apex, the basal edge of zone 3 being at this point. The growth rate falls off very quickly at the end of zone 3. As appears later, the rate decreases from its maximum to zero in probably less than half an hour, the time interval between measurements. Therefore, it was not possible to determine here either the point at which the decline in growth rate first began or the length of the region in which it occurred. Since the measurement was made

at the time that elongation ceased, the region in which the decline in growth rate occurred is included in zone 3.

The results of similar measurements for complexes 5, 8, 11, 16, and "31-32" are shown in table 1. The measurements were difficult to make accurately since the times at which the changes in growth rate occurred could not be determined exactly. However, the agreement between measurements for different complexes is fairly close.

The relative lengths of the different zones and the growth rate in each is made clear by reference to the diagram shown in figure 6. The graph shows the growth rates of cells and their resultant complexes, expressed in per cent per hour, plotted against their distances from the root apex. Ordinates are growth rates, and abscissae are distances from the apex (root cap not included). Solid lines indicate growth rates as determined from the growth curves. Dotted lines indicate estimated rates in regions where it was impossible to obtain an accurate determination. As seen from the graph, all cells within a zone extending from the root tip to about 0.14 mm. behind it grow at an exponential rate of about 5.4 per cent per hour. In the next 0.17 mm. (from 0.14 mm. to 0.31 mm.) the rate increases from 5.4 per cent per hour to about 41.2 per cent per hour. Cells in the next 0.66 mm. (from 0.31 mm. to 0.97 mm.) grow at a constant rate of about 41.2 per cent per hour. The diagram of the root tip below the graph is drawn to the same scale as the abscissae of the graph, and it shows the relative proportions of the three growth zones. Cell division occurs only in zone 1 under normal conditions.

Each cell becomes farther and farther removed from the root apex because of the elongation of intervening cells. As its distance from the root apex increases, the cell passes through zones 1, 2, and 3, successively. When the cell passes over the basal edge of zone 3, it ceases to elongate and becomes a part of the mature root. The length of each zone is apparently constant (table 1), and the growth rate in each zone evidently remains constant, since the growth curves for all complexes are similar (fig. 5). The rate at which cells pass out of zone 3 and become a part of the mature root must also be constant, since this rate could vary only as a result of changes in the length of the growth zones or variations in the cell growth



rates. Since root growth results only from the activity of the growing point, the rate of root elongation should be the same as the rate at which cells pass over the basal edge of zone 3.

The rate at which cells pass over this point and reach their final size could readily be determined by reference to the growth curves shown in figure 5 and measurements made from the photographic record. Complex 31-32 ceased growth at about 5:30 P.M., June 21 (fig. 5), indicating that the apical end of the complex had just passed through zone 3 at that time. Complex 10, which was nearer the root apex than 31-32, stopped elongating at 4:00 A.M., June 22, indicating that its apical end had then just passed through zone 3. Thus, ten and one-half hours were required for the length of cell wall between the apical end of complex 31-32 and the apical end of complex 10 to pass out of zone 3. The distance between these two points, measured from photographs taken at the time it had reached its final length, was 2.76 mm. Thus cells passed over the basal edge of zone 3 at a rate of 0.263 mm. per hour, this being the rate at which mature cells were added to the length of the root as a whole.

The results of the measurements of root growth are shown graphically in figure 7, root length being plotted against time on an arithmetic grid. The figure shows that total root length increased at a constant rate of about 0.266 mm. This agrees very well with the rate at which cells passed over the basal edge of zone 3 (0.263 mm. per hour), considering possible error in determining the two rates. The constant rate of root elongation shows that the growth rate in each zone and the length of each zone remained remarkably constant for the duration of the experiment, since variation in either would obviously influence the rate of root elongation.

The origin of cells of different size, such as trichoblasts and hairless cells, is of especial interest in this study. Such cells were formed in complex 11 by unequal divisions which are almost always the final ones (fig. 3C, cf. Sinnott and Bloch, 1939a). The contents of the trichoblasts are more dense and their basal transverse walls are convex towards the base of the root (cf. Leavitt, 1904; Sinnott and Bloch, 1939a).

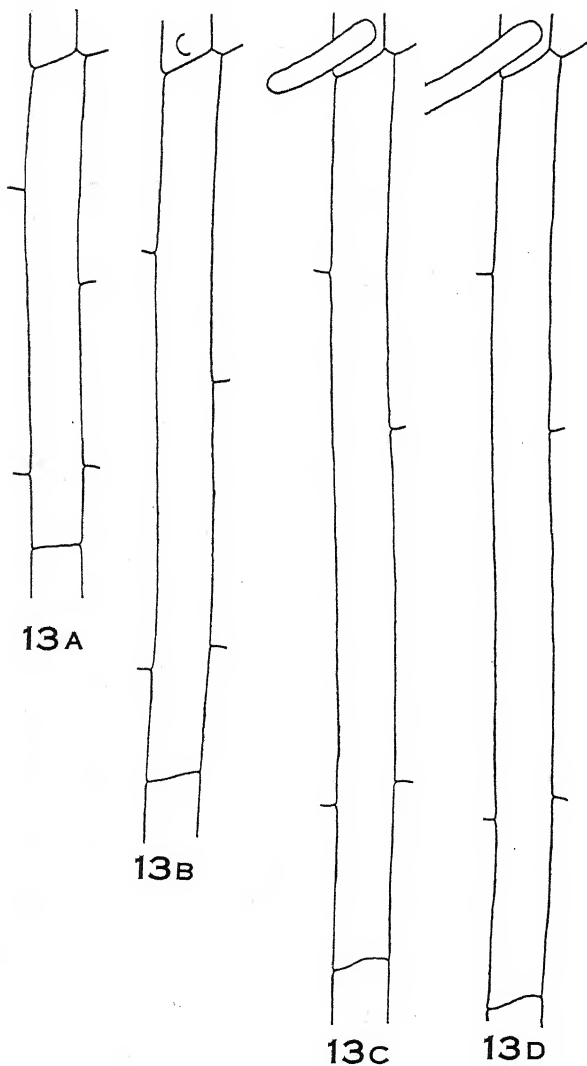
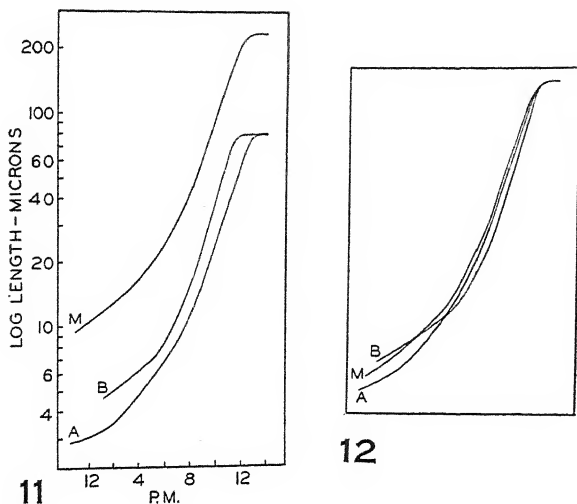
Fig. 4-10. Growth of cells in a living grass root.—Fig. 4. Growth curve for a single epidermal cell and its descendants as it passed from the meristematic condition to maturity (cell number 11 in figures 3 A-E). Logarithmic plotting. Arrows indicate time of division.—Fig. 5. Growth curves for six epidermal cells and their descendants located at different distances from the apex of the root. The cells and their resultant complexes are numbered as in figures 3, A-E. Cell 5 was nearest the apex and "31-32" farthest from it. Cells 5 and 8 did not complete their growth during the period of observation. Logarithmic plotting.—Fig. 6. Diagram showing the growth zones observed in a living root of *Phleum pratense* and the growth rates of epidermal cells in each zone. The graph shows the growth rate of the cell plotted against its distance from the root apex. Solid lines indicate the actual rates as determined from growth curves; dotted lines show the estimated rates in regions of the root where it was impossible to obtain an accurate determination. Below the graph is a diagram of the root drawn to the same scale as the abscissae of the graph. In zone 1 the growth rate remains constant at about 5.4 per cent per hour, in zone 2 it increases to about 41.2 per cent, and in zone 3 it remains constant at 41.2 per cent, falling off to zero in the basal end of zone 3.—Fig. 7. Growth curve for a living root of *Phleum pratense*. Arithmetic plotting.—Fig. 8. Growth curves for each of the four descendants of a single epidermal cell which formed trichoblasts and hairless cells (cell 11 in figures 3, A-E). 11a and 11c are trichoblasts while 11b and 11d are hairless cells. Logarithmic plotting.—Fig. 9. Growth curves for each of the four descendants of a single epidermal cell which did not form trichoblasts (cell 10 in figures 3, A-E). Logarithmic plotting.—Fig. 10. Growth curves from figures 8 and 9 superimposed for better comparison. The curves are superimposed in such a way that the points at which growth ceased coincide.

The unequal division is largely responsible for the smaller size of the trichoblasts but it also appears that they elongate less than the hairless cells. Growth curves were plotted for the individual cells of complex 11 so that growth in the two types of cells could be compared (fig. 8). Growth curves for the individual cells of complex 10, none of which became trichoblasts, were also plotted (fig. 9). Measurements were not begun until two hours after their formation, since new cell walls do not show up well in the photographs. The last divisions in both complex 10 and 11 occurred near the end of the first growth phase, and consequently the growth curves for the individual cells of each complex represent little of the first growth phase.

As is evident from figure 8, the trichoblasts did not elongate as much as the hairless cells (cf. Sinnott and Bloch, 1939b). After the unequal division, cell 11A was one-third as long as its sister cell 11B, but after reaching their final size cell 11A was only about one-sixth the length of 11B. Similarly, cell 11C was about two-fifths the length of its sister cell 11D at the time of its formation, but after growth was completed, cell 11C was about one-third the length of 11D. All four daughter cells elongated nearly the same amount in complex 10.

The growth curves for the individual cells of complex 11 and complex 10 were superimposed in such a way that the points at which growth ceased coincided (fig. 10). The curves for all the cells of complex 10 and for the hairless cells of complex 11 are quite similar, falling within a rather narrow band. The trichoblasts evidently differ from the others in that they tend to grow more slowly during the transition period, and that for them the second growth phase is of somewhat shorter duration.

Nothing indicating the occurrence of sliding growth has been observed during the course of this investigation. If cells in adjacent rows were to slide along one another during growth, then transverse walls of cells in adjacent rows should occasionally move past one another. Also, the distance between transverse walls in adjacent cell rows should be observed to decrease in some cases. Transverse walls in adjacent rows were never seen to move past one an-



other and the distance between such walls always increased. This is strong evidence that "sliding growth" does not occur in this tissue (*cf.* Sinnott, 1939; Sinnott and Bloch, 1939b).

If the walls of neighboring cells do not slide along each other, then the points at which end walls of cells in adjacent rows meet the longitudinal wall of a given cell can be used as marker points to divide it into smaller units. In this way growth curves can be plotted for different parts of a single cell and growth in these parts compared. Cell 11D, a long hairless cell, was chosen for this purpose. It was divided into apical, middle, and basal portions which were marked by end walls in complex 10, all cells of which were hairless ones (fig. 3D). This choice was made to avoid as much as possible any influence of neighboring trichoblasts. The basal part of cell 11D consisted of that part of the cell wall extending from the basal end wall of cell 11D to the basal end wall of cell 10C. This part is the same as the apical portion of cell 10D. The middle part of cell 11D was marked by the end walls of cell 10C and its growth curve is of course the same as that for cell 10C. The apical part of cell 11D consisted of that part between the apical end wall of cell 10C and the apical end wall of cell 11D, this being the same as the basal part of cell 10B. Measurements were begun when the cell walls became sufficiently well defined to show clearly in the photographs, about two hours after the last division, so during most of the period of measurement the cell was in the transition period and the second growth phase.

Growth curves for the three parts of cell 11D are shown in figure 11 and are superimposed in figure 12. The apical end of the cell evidently grew somewhat more than the middle part, and the middle part somewhat more than the basal end, although the differences are slight. The differences in the curves are greatest in those parts representing the transition from the first phase to the second, while those parts of the curves representing the second growth phase are essentially parallel. Intercellular relationships which may have affected the growth of different parts of cell 11D could not be determined, since rotation of the root made it impossible to study the growth of cells in rows adjacent to complex 10 and

Fig. 11-13D. Growth of different parts of the same cell. —Fig. 11. Growth curves for different parts of a single cell (cell 11d in figure 3D) marked by end walls of a cell in an adjacent cell row (cell 10 in figure 3D). A, is the apical part; M, the middle part; and B, the basal part. Logarithmic plotting.—Fig. 12. Growth curves from figure 11 superimposed for better comparison. The curves are superimposed in such a way that the points at which growth ceased coincide.—Fig. 13, A-D. A single cell (11D in figure 3D) drawn at one-hour intervals covering the period from the time that all parts of the cell were growing at the maximum rate until the cell reached its final size. Different parts of the cell are marked by cross walls in adjacent cell rows. The apical end of the cell is toward the bottom of the figures. A, June 21, 11:30 P.M. B, June 22, 12:30 A.M. C, June 22, 1:30 A.M. D, June 22, 2:30 A.M. Traced from the photomicrographs. X 313.

complex 11. It is clear, however, that differential wall growth does occur and that different parts of the same cell may be growing at quite different rates at the same time. Thus, as seen from figure 11, the basal end of cell 11D completed its growth at about 12:00 midnight, June 21, while the apical end of the same cell was still growing at its maximum rate. This differential growth of the cell can be seen more clearly in figures 13A-D which were traced from photographs taken at one-hour intervals covering the period from the time that all parts of the cell were growing at the maximum rate until the cell reached its final size; i.e., from 11:30 P.M., June 21, to 2:30 A.M., June 22. The series shows that the basal end stopped growing first, the middle part next, and the apical end last, the cessation of growth passing along the cell from base to apex (cf. Sinnott and Bloch, 1939b).

Figure 11 shows that the growth rate of each part of the cell fell off to zero abruptly, doing so in the case of middle part of cell 11D in about one hour. This time interval is about the same as that required for this part of the cell to pass out of zone 3, since cells pass out of this zone at about 0.263 mm. per hour, and since the middle part of cell 11D was about 0.22 mm. in length when it reached its final size. A similar agreement between the time required for the cell or its derivatives to pass out of zone 3 and the length of the period of declining growth rate can be shown for all cells measured. This being so, the decline in growth rate seen at the ends of the growth curves shown in figures 5, 8, 9, and 11 evidently results primarily from the fact that shorter and shorter lengths of the cells or complexes are growing as they pass out of zone 3, rather than to a lower rate of growth in the basal part of the zone. Thus each cell evidently grows at its maximum rate up to very near the end of zone 3, the growth rate falling off to zero in a very short region. The suddenness with which growth ceases evidently has an important bearing on the factors controlling cell growth. It was impossible however, to determine how rapidly the decline took place, the interval between successive measurements being too long.

Similar results were obtained in an earlier experiment using the same technique, also on *Phleum pratense*. The photographs were taken at two-hour intervals, but unfortunately they did not include a sufficient length of the root apex to allow development to be followed until the cells reached their final size; i.e., only a part of zone 3 was included in the photographs. In zone 1 the rate was constant at about 6.5 per cent per hour and in zone 3 at about 56 per cent per hour. The root as a whole elongated at about 0.39 mm. per hour. Zone 1 extended to about 0.16 mm. behind the root apex (root cap not included). The higher rates observed in the earlier experiment were probably not due to differences in environmental conditions, which were about the same in the two experiments, but to genetic or possibly size differences in the two seedlings.

DISCUSSION.—The results reported here evidently have an important bearing on such problems as the growth processes in the root apex; growth regions in the root meristem; differential cell growth and cell differentiation; rhythmic growth of organs and cells; the physiology of cell elongation (cf. Heyn, 1940); and the differential effect of heteroauxin on root elongation and meristematic activity in producing lateral roots (cf. Albaum and Commoner, 1941).

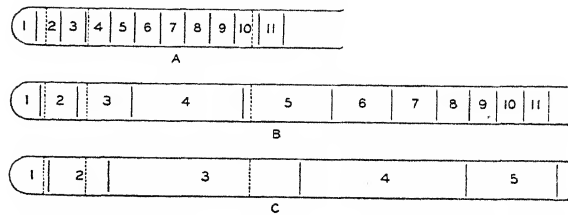


Fig. 14, A-C. Diagrams showing the amount of growth made by 0.1 mm. sections of a *Phleum pratense* root. A shows the position of the sections at the time of marking, while B and C show the amount of growth made after 4 and 7½ hours respectively. Dotted lines indicate the three growth zones as shown in figure 6.

Space does not permit a discussion of all these aspects however, and the discussion will be limited to those problems to which the present investigation is more directly related.

Growth processes.—Two distinct processes are evidently involved in growth of this tissue. During the first phase of growth the cell elongates at a relatively low but constant exponential rate. Divisions occur during this phase, dividing the cell into a complex of smaller units, but without markedly influencing the growth rate of the cell or its resultant complex. As the cell or complex grows, it becomes farther removed from the root apex because of the elongation of cells apical to it. When the cell reaches a distance of about 0.14 mm. behind the root apex, cell division ceases, and the first growth process begins to be supplanted by a second and more rapid process of elongation, the growth rate gradually increasing until the new constant rate is established. During the period of increasing growth rate the vacuoles within the cell become larger and coalesce. This continues during the second growth phase, and finally a single large vacuole fills the center of the cell, and the cytoplasm extends as a thin sheet next to the cell wall. Thus it appears that in the first growth process cell elongation results primarily from the production of new protoplasm accompanied by the formation of new cells, while in the second process the cell elongates primarily as a result of increase in volume of its vacuoles and the formation of new cell wall material (cf. Priestley, 1929). The period of increasing growth rate apparently represents a transition from one process to the other.

That new protoplasm is formed primarily in a region close to the tip is also suggested by the work of Reid (1941) on cowpea roots. In these it was found that throughout growth the water content of the cell

is proportional to its volume, while its dry weight increases most rapidly in a region close to the root apex.

Growth regions.—In the older method used to detect regions of most rapid growth, the growing point is marked with India ink at regular intervals along its length. The transverse section thus marked off, which later shows the most growth, indicates the region of greatest elongation. In a typical experiment of this type, Sachs (1882) made transverse marks at one millimeter intervals behind the apex of a primary root of a *Vicia faba* seedling. Twenty-four hours later it was found that the greatest elongation had occurred in a one-millimeter section lying about 3 mm. behind the root apex. It was concluded from this that the region of most rapid growth is a rather short one.

As seen from figure 6, the region of most rapid elongation in *Phleum pratense* occupies most of the length of the growing point. It can be shown, however, that this is not in disagreement with the results obtained by the marking experiments. Photographs taken at 9:30 P.M., June 21, were marked with transverse lines, the distance between the lines being such as to correspond to 0.1 mm. on the growing root. It was possible to determine in photographs taken at later periods the amount of growth made by each 0.1 mm. section, using cross walls as reference points. Essentially, this amounts to duplicating Sachs' marking experiment in a much more accurate manner. The position of each 0.1 mm. section marked off at the beginning is shown diagrammatically in figure 14A, and the length of each section after four and seven and one-half hours is shown in figures 14B and 14C respectively. Dotted lines indicate the growth zones as shown in figure 6.

Four hours after marking, section 4 had made the greatest growth, since it grew by the higher rate in zone 3 for a longer period than the others. Section 5 elongated less than section 4, since it remained in zone 3 for a shorter time. Similarly, section 6 was shorter than 5, 7 shorter than 6, etc., and section 11 showed no growth, since it had completed elongation at the time of marking. Section 3, being in zone 2 at the time of marking and, consequently, elongating at a lower rate than section 4, did not grow as rapidly as section 4 during the four hour period. Therefore, it was shorter than section 4. Seven and one-half hours after marking, however, section 3 was the longest, since it grew for a longer period than 4, while section 2 was just beginning to grow by the higher rate in zone 3. Sections 6, 7, 8, 9, and 10 were no longer after seven and one-half hours than after four hours, since they passed out of zone 3 during the first four hours. Obviously, if measurements were made still later, section 2 would be the longest. The amount of growth made by sections marked off in the manner described evidently depends on the position of the section with respect to the growth zones and on the time elapsing between marking and later measurement. Thus, this type of experiment is of little value in detecting growth in different regions of

the growing point unless measurements are made at frequent intervals.

Differential cell growth.—The differential growth of trichoblasts and hairless cells is of especial interest, since it has a bearing on the problem of cell differentiation. The results of this investigation show that the difference in growth rate in the two types of cells is greatest in zone 2, where a transition from protoplasmic synthesis to vacuole enlargement apparently occurs. The trichoblasts, which are more richly protoplasmic, continue growth in zone 2, at more nearly the rate of growth which occurs in zone 1, while the growth rate of the hairless cells increases. Associated with the differences in growth is the fact that vacuoles appear in the hairless cells sooner than in the trichoblasts. The differences in behavior of the two types of cells may be due to differences in the structure of their walls, since, as Sinnott and Bloch (1939b) have shown, the walls of the trichoblasts appear to be more rigid than those of the hairless cells. It is also possible that the trichoblasts continue protoplasmic synthesis for a longer period than the hairless cells. This would account for the slower increase in growth rate of the trichoblasts and for the earlier appearance of large vacuoles in the hairless cells. As a third possibility, it may be that growth by vacuolation is induced by some stimulus passing from the base of the root and that the trichoblasts and hairless cells respond to the stimulus differently because of differentiation occurring at the time of the unequal division. Although no conclusions can be drawn at the present time as to why the two types of cells differ during growth, it is clear that the difference in growth rate appears immediately after the division forming them. As evidenced by the curves in figures 11 and 12, later growth in both types of cells is very nearly the same.

Rhythmic growth.—Kellicott (1904) working with germinating bulbs of *Allium cepa* reported that root elongation was rhythmic, exhibiting three waves of elongation in a twenty-four-hour period. Friesner (1920) also observed rhythms of elongation in roots of a variety of plants, most comprehensive results being reported for *Pisum sativum*. Two or more waves per twenty-four-hour period were reported, periods of maxima and minima being associated with the time of seed germination. Cell division showed similar rhythms, maxima falling at periods of minimum root elongation and *vice versa*. It was concluded from this that the energy of the growing root was directed at one time toward cell division and at another toward cell elongation. It is evident from figure 7 that, in the root observed here, no rhythms of elongation were exhibited by the root as a whole, and from figure 4 that the growth of the cells is not influenced by their division but is continuous at a constant rate. It is possible that here the periodic lighting of the root for photography may have caused responses which masked any tendency toward rhythmic growth. Yet it appears, *a priori*, that rhythms in cell division should have little effect on the rate of root elongation, since division occurs only in a region

whose growth adds but little to root length (cf. Kojima, 1928). The greater part of root elongation results from growth in length of cells in zone 3 where cell division does not occur. As seen from figure 5, elongation of cells in this region is not rhythmic, all cells in this region growing at the same constant exponential rate.

Abele (1936), in a statistical study based on measurements of cells in fixed material, concluded that in meristematic tissue cell growth occurs in two stages. Shortly after telophase, "Streckungswachstum" occurs during which the cell increases to the average size of the resting cells. No growth occurs during the resting stage. "Teilungswachstum" takes place following the resting stage, the cell reaching its maximum size by late prophase and no further growth occurring until after division is completed. It is clear from figure 4 that in the living root observed here no growth rhythms were detected, cell elongation continuing at a constant rate regardless of its division. Moreover, the behavior described by Abele would obviously involve sliding growth, which apparently does not occur in this tissue.

SUMMARY

Phleum pratense seedlings were grown in a specially constructed moist chamber, and groups of meristematic cells in the root tips were photographed at intervals as they developed to maturity. Growth curves of individual cells and cell complexes resulting from their division were plotted from measurements of cell length made from the photographs.

Each cell or its resultant complex passes through

two phases of growth, elongation proceeding at a constant exponential rate in each. In the first phase, the cell or complex elongates at about 5.4 per cent per hour. At the end of this phase, the growth rate increases from 5.4 per cent per hour to about 41.2 per cent per hour. In the second phase the rate remains constant at 41.2 per cent per hour, the growth rate falling off to zero at the end of this phase. Cell division occurs only during the first phase.

The first phase of growth appears to be primarily concerned with the production of new protoplasm and the second with vacuole enlargement. The period of increasing growth rate apparently represents a transition from one growth phase to the other.

The first phase occurs in a zone extending from the root tip to 0.14 mm. behind it (root cap not included). The transition occurs in a second zone which extends from 0.14 mm. behind the root tip to 0.31 mm. behind the tip. The second growth phase takes place in a zone extending from 0.31 mm. behind the root tip to 0.97 mm. behind it. In the basal end of this zone the growth rate falls off to zero rapidly.

Trichoblasts elongate less than hairless cells. The growth rate of the trichoblasts increases more gradually than that of the hairless cells during transition from protoplasmic synthesis to vacuolation.

No evidence of "rhythmic" growth was observed in root elongation, and the process of division did not appear to influence the rate of cell elongation.

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CATECHOL AGGREGATES IN THE VACUOLES OF CELLS OF ZINC DEFICIENT PLANTS ¹

Howard S. Reed and Jean Dufrenoy

CELLULAR INCLUSIONS have, for over a century, received attention from plant cytologists. The results of many investigations which employed either chemical or physical criteria agree that colloids of one sort or another are present in plant cell vacuoles. Evidence of their physiological importance which is now at hand promises to elucidate some phenomena having great significance for the activity of the cell. Precise studies have shown that differences in their states of hydration and dispersion are intimately related to health and disease. They are also plainly important in determining the water-holding capacity, the absorption of "vital dyes," and the viscosity of the cell sap.

The present paper presents new data on the formation of catechol-lipoidic aggregates in the vacuolar sap and shows how they are related to certain nutrient deficiencies of plants. Although phenolic materials of this type have long been known in many plants, their relation to the physiology of the cell has been imperfectly understood. On account of their small size and their indifference to the majority of aniline stains, bodies of this class have been frequently overlooked or wrongly identified as parasitic myxomycetes, chytrids, or other organisms.

Viala and Sauvageau who, in southern France, investigated the vine disease, known as Brunisure, described (1892 a and b) the cell inclusions as plasmodia of a myxomycete. Prunet, who designated (1894) similar inclusions as chytrids, e.g. *Cladochytrium viticolum*, initiated, apparently, a chytrid fad which prevailed for several years and subsequently reappeared from time to time. Although these identifications as organisms were wrong, they did establish the important fact that these vacuolar inclusions were associated with a diseased condition in the plant. Massee, who had originally succumbed to the chytrid fad, realized eventually (1895) that the inclusions previously described as chytrids were "tannin vesicles" of the sort described by Klercker (1888) and opened the road for further studies.

Ducommet (1900) corrected the erroneous idea of a plasmodial parasite in vines affected with Brunisure by showing that the vacuolar inclusions were aggregates of material which previously had been dispersed in the vacuolar sap. He observed that cells in the vine leaves affected with Brunisure contained both small and large spherical bodies and that they might coalesce. They had enveloping membranes and their contents might appear homogeneous or vacuolated. His microchemical tests showed that they were strongly impregnated with tannin, insoluble in fat solvents, and soluble in sodium hypochlorite.

The occurrence of globular inclusions of this type in the cell vacuoles of plants affected by nutrient

deficiencies was slow in receiving recognition. We have called attention (Reed and Dufrenoy, 1934 and 1935) to globular refringent inclusions of this type in cells of orange leaves showing mottling due to zinc deficiency. These vacuolar inclusions showed a certain amount of heterogeneity, having a central core of phenolic material and an enveloping layer of phytosterol material. We assumed the latter to represent an unused residue in those cells whose synthetic power was seriously impaired. We subsequently showed that treatments which restored the leaves to a healthy condition were characterized by the absence of those globular refringent inclusions.

Coacervates of this sort are associated with virus disease of plants. Dufrenoy (1942) has described them in the perivascular parenchyma cells of sugar cane affected with Chlorotic Streak, as well as in senescent canes.

Vacuolar aggregates of lipoidal nature occur in root cells invaded by endophytic mycorrhizas (McLuckie and Burges, 1932). If the globules possessed a central core of non-lipoidic material, possibly catechol (as their reactions suggest), further investigation should yield interesting results because of their importance in the physiological reactions of infected cells. The state of nutrition of the mycorrhizal cell has an intimate relation to the formation of vacuolar aggregates of phenolic material, as demonstrated by photomicrographs of cells which came from the roots of orange trees showing definite responses to soil treatments (Reed and Frémont, 1935). The aggregates of phenolic material occurred chiefly in cells in which the fungus lived parasitically rather than symbiotically.

The formation of these inclusions as a reaction to invasion by parasitic organisms has been recently demonstrated by Metcalfe (1941) who has described vacuolar inclusions (Y-bodies) in the ray-cells of willows attacked by parasitic bacteria. Although he misinterpreted certain details, he saw aggregates similar to those we have studied, if one may judge from his illustrations and descriptions of their reactions to micro-chemical reagents. Dufrenoy (1942) has described catechol aggregates of this type in cells at the margins of lesions produced in sugar cane by *Colletotrichum falcatum*.

It is now apparent that inclusions of catechol are related to at least three types of functional derangements, namely: (1) a deficiency of nutrients (using the term "nutrient" in a broad sense); (2) virus diseases; (3) attacks of parasitic organisms.

Cells of leaves showing symptoms of zinc deficiency commonly exhibit a condition in which the colloids of the vacuolar sap occur as flocculi floating in the liquid phase. The flocculi have a tendency to form globular aggregates which, on account of their uniformity, have been mistaken for micro-organ-

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isms; however, they may coalesce to form larger aggregates. The formation of colloidal globules by a process of desolvation or "unmixing" may be readily observed when living cells of affected plants are examined in neutral red solution. Figure 1 shows an example wherein the globules (represented by heavy shading) have separated out from the central vacuolar solution (represented by light shading) by the

sap. In the upper drawing we see the result of "vital staining" with neutral red or methylene blue. The positively charged dye accumulated in the spherical mass of colloidal material which shows a deeply stained ring. In the lower drawing we see the result of the application of Gomori's technique for PO_4^- anions. The negatively charged anions were dispersed throughout the saline vacuolar solution

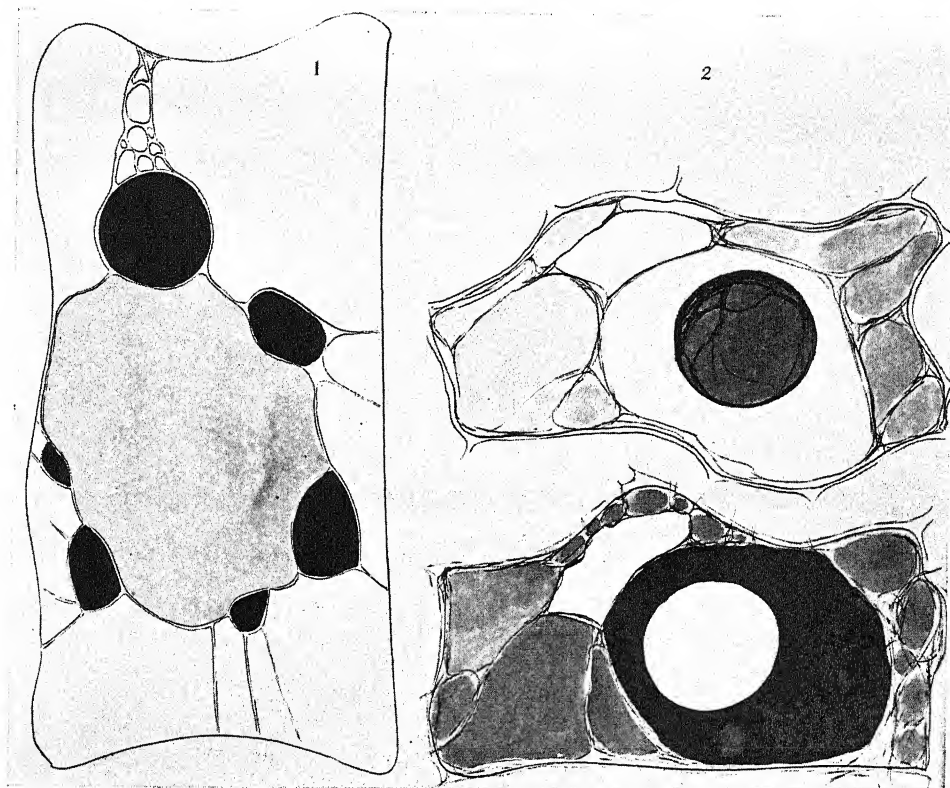


Fig. 1-2.—Fig. 1. A representation of a living cell, stained with neutral red, featuring a case of syneresis, where the central vacuolar solution (featured light grey) is surrounded by a number of peripheral smaller vacuoles, wherein the colloidal material responsible for the absorption of neutral red has become concentrated, accounting for the much deeper staining of the solution (featured black).—Fig. 2. A schematic representation of the distribution of cations and anions in a vacuolar solution after syneresis and condensation of the colloids into a globular mass. The upper drawing features a living cell after being vitally stained with a basic dye. The lower drawing features a cell after being treated with Gomori's technique for PO_4^- . For further comment see text.

process of syneresis. When the process of desolvation affects only a portion of the colloidal material in the vacuole, it results in the formation of a coacervate instead of a generalized syneresis.

The formation of vacuolar precipitates when a cell is placed in contact with "vital dyes" is considered by Guilliermond (1941), not as flocculation, but as the production of coacervates due to the attraction between the positively charged dye and the negatively charged colloid. The anions are concomitantly dispersed in the vacuolar sap. The phenomenon is illustrated diagrammatically by figure 2, which represents the state of the vacuolar colloids following inner syneresis and the formation of a large spherical mass which floated in the vacuolar

(heavily shaded), whereas the central mass of colloidal material was devoid of PO_4^- ions.

Recent advances in cyto-technique and in micro-physical chemistry have afforded a vast amount of new information about these vacuolar inclusions (Dufrenoy and Reed, 1942). The papers on the cytological significance of these complex aggregates by Guilliermond and his co-workers and by de Jong (1929) on their physico-chemical significance have contributed additional evidence of their importance. The results of our subsequent work, which are reported in this paper, demonstrate that these complex bodies, called coacervates or aggregates, occur in the post-meristematic cells of root-tips, buds, and leaves of plants whose development was retarded owing to

a deficiency of zinc. These coacervates were seldom found in healthy plants. This work was done by the technique which Guilliermond has called "convergent technique," together with certain modifications which the authors have worked out and will describe below. Our observations agree well with the data on coacervates published by de Jong and on the reactions of catechols with vital dyes published by Guilliermond.

It seems evident from the works just cited that these bodies are formed by a process of coacervation which we define as a process in which some colloidal material in the vacuolar solution of the cell becomes condensed into a spherical mass, instead of being distributed at random in the water. This spherical mass floats in the vacuolar solution, in which the colloidal particles were formerly dispersed. Its formation is a simple consequence of a gradient in the distribution of cations and correlative distribution of polyphenol oxidase (a copper protein). Although the process of coacervation may to a certain extent be visualized as a separation of phases, the boundary layer is more than the theoretical interphase; it is a precipitation membrane composed of oriented molecules.

The presence of catechol in the central mass of the coacervate is revealed by the brown color developed upon oxidation as well as by its selective absorption of neutral red (Dufrenoy and Reed 1942). Coacervates, therefore, may be defined as centers of high polyphenol oxidase activity with a definite boundary where the oxidizing activity is at its maximum. That boundary is the interphase between the colloidal central mass, in which oxidase activity can be detected, and the surrounding vacuolar liquid in which no such activity can be revealed. The active migration and concentration of basic dyes into the coacervates, and still more conspicuously in the precipitation membrane, are correlated with the aforementioned distribution of polyphenol oxidase. Data to be presented in this paper show that plant cells suffering from certain mineral deficiencies build up accretions wherein the colloidal catechol compound of the vacuolar solution becomes condensed into a concentrated globular mass, and forms a surface film of phospho-lipoids through a molecular orientation at the interphase. This idea is experimentally supported by the fact that catechol reactions may be obtained from the central mass of the globule, and reactions both for fats and phosphorus compounds may be obtained from the surface precipitation membrane (Dufrenoy and Reed, 1942).

It seems appropriate to allude to the relation of the oxidase to permeability and exchange of ions though the phenomenon cannot be here discussed in detail. Raciborski (1898) called attention to the high concentration in the phloem elements of what we now know as indophenol and catechol oxidase systems. Boswell and Whiting (1938), who investigated the oxidase system of potato tubers, concluded that the catechol oxidase is responsible for the major part of the O_2 uptake and of the CO_2 released. The

fundamental importance of the phenol-oxidase for the cell has been emphasized by Hoagland's discovery (1923) that the absorption of solutes is closely dependent upon the energy exchange, and has been studied further by Steward, Stout, and Preston (1940) who showed that respiration of potato disks is partially linked to a respiratory component which was influenced by salts and by oxygen concentration. As the oxidase system plays an essential rôle in cell respiration, it may be expected to play a similar rôle in permeability and water uptake.

TECHNIQUE.—Neutral red and methylene blue are especially useful for *intra vitam* demonstration of catechols in plant cells, though Nile Blue and similar dyes also gave differentiation, part of the dye being absorbed by the central phenolic core and part by the fat in the outer layer. The presence of catechol oxidase was demonstrated by the convergent techniques: (A) post-vital staining and (B) vital staining.

(A) We have used two post-vital staining methods: (1) the classical indophenol blue reaction and (2) the 2-6-dichloro-quinone-chloroimide, a reagent recently introduced as a test for vitamin B_6 which is also very useful as a test for catechol oxidase. The indophenol blue was obtained, as usual, from the neutralized equimolecular mixture of dimethyl paraphenylene diamine and thymol (or alpha naphthol), these mixtures being, for the sake of brevity, designated as thydi or nadi. Upon exposure to the atmospheric oxygen, either of these substances will slowly form phenol blue, but, in the presence of indophenol oxidase, the blue color forms more quickly. With catechol oxidase in the system, however, the formation of indophenol blue is catalyzed indirectly via oxidation of catechol to quinone. Catechol oxidase, being a copper protein, is cyanide sensitive. The 2-6-dichloro-quinone-chloroimide is insoluble in water but soluble in a solution of catechol. The red color, deepening to brown, developing on tissues immersed in a watery suspension of the reagent affords a precise localization of catechol oxidase. This reaction also is cyanide sensitive, as it involves a copper protein. Obviously, the relation of this to metals such as zinc should be investigated.

(B) We have tested the following dyes on free-hand sections of plant material: safranin, thionine, methylene blue, basic fuchsin, neutral red, quinoleine blue (cyanine), rhodamine, and chrysoidin and demonstrated that they accumulated in the coacervates, staining the external membrane more deeply than the central core.

It is assumed that anions (for instance, acid dyes) should be carried from a region of high concentration to one where the concentration is low; and conversely that cations (for instance, basic dyes) should accumulate at the foci of the oxidase system.

We have obtained evidence from cytological observations (Reed and Dufrenoy, 1935) that the blue granules of indophenol blue which form more rapidly at some foci within the vacuolar solution are centers of catechol oxidase activity. Where the colloidal

catechol compound had formed spherical masses, the application of other reagents for oxidase demonstrated that their core is not only the focus for catechol derivatives, but also for catechol oxidase (Dufrenoy and Reed, 1942). Therefore, we should expect a difference of electrical potentials to prevail between the coacervate and the surrounding medium, with a resultant tendency for cations to move into the coacervate through the surrounding membrane. Obviously, this would have a great influence upon the intake of dissolved material by the living cell. On account of the irreversibility of the process the coacervates would trap cations from the vacuolar solution.

The indophenol or catechol oxidase in the presence of the thydi mixture develops a brown color in the phenolic core of the coacervate and a blue color in the lipoidic membrane surrounding it. In this membrane the nascent indophenol blue becomes preferentially dissolved.

The molybdenic reagent, described below, is still more valuable for work on these coacervates. It causes a browning of the inner phenolic core and the formation of blue wherever phosphoric acid is present. Since the enveloping membrane contains phospho-lipoids, it is colored blue by this reagent. It can be applied either to freehand sections of such material, since its strong oxidizing effect produces very efficient fixation, or it can be applied to microtome sections of material previously fixed with good killing agents such as Nemec's or Helly's fluid. Sections treated with the molybdenic reagent may then be stained with haematoxylin or with acid fuchsin and checked against sections of homologous tissues stained directly with those dyes.

The molybdenic reagent for phosphorus compounds is the conventional test for thiamin. It is made as follows:

Add 20.9 cc. of concentrated sulfuric acid to enough water to make up 250 cc. of 3 N solution of sulfuric acid, in which 6.41 g. of ammonium molybdate is dissolved. This constitutes Solution A.

Dissolve 5.75 g. sodium bisulfite in 85 cc. distilled water. Add .5 g. of 1-amino 2-naphthol 4-sulfonic acid and 5 cc. of 20 per cent sodium sulfite solution. Stir the mixture until the sulfonic acid dissolves. This is Solution B.

Slides bearing microtome sections of plant tissues should be placed in a staining jar containing a mixture of 25 cc. of solution A and 5 cc. of solution B. The slides should remain in this for several days until the sections develop a deep blue color. They can then be dehydrated rapidly by washing with 95 per cent alcohol and then mounted in diaphane. As the result of this treatment, nucleoli and prochromosomes, plastids and mitochondria show a blue color. The blue color may be intensified to very dark blue or deep purple by immersing the slide, after it is taken out of the molybdenic solution, for one minute in an aqueous 1 per cent solution of haematoxylin previous to dehydration. However, if one wishes to differentiate mitochondria from plastids, the section can be stained with acid fuchsin and counterstained

with an alcoholic solution of aurantia. Plastids, which then retain a blue-purple color, are beautifully differentiated from mitochondria, which appear bright red.

VACUOLAR INCLUSIONS.—The data which we herewith present have been obtained since the publication of our earlier work and afford additional information concerning the effects of zinc in maintaining the physiological equilibrium of the cell.

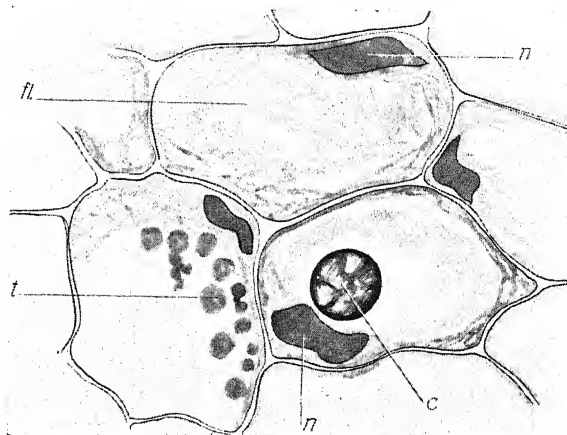


Fig. 3. Cells from the post-meristematic region of an affected apricot shoot, in which the nuclei (n) were appressed along the cell walls. Three types of catechol aggregates were found; a fluffy mass (fl) distributed through the entire vacuole, globular precipitates (t) generally at the periphery of the vacuole, and spherical masses (c) enveloped by precipitation membranes, *i.e.*, coacervates.

An important aspect of the phenolic precipitates is shown in cells (fig. 3) from the post meristematic region of an apricot shoot affected with little-leaf. The material had been collected July 29, at a time when growth for the season had practically ceased and when the cells contained a maximum of phenolic materials, as previously described (Reed, 1941). The preparation was made from material fixed in Nemec's solution, and the sections were stained with acid fuchsin followed by methyl green. The nuclei (n) were usually in a parietal position surrounded by a thin film of the abnormally vacuolate cytoplasm. The large central vacuoles contained inclusions of several sorts. The catechol materials in the upper cell had formed a diffuse flocculum (fl) which occupied most of the vacuolar space. They formed numerous spherical precipitates (t) in the cell at the left of figure 3. The catechol materials in the cell at the right formed a coacervated aggregate (c) which contained most of the colloids from the vacuolar solution and was enveloped by an alveolate layer. The vacuole also contained a flocculent precipitate of phenolic material. The diffuse flocculum which the upper cell contained resembled a condition found (Reed, 1938 b) in palisade parenchyma of an apricot leaf which had grown in a culture solution lacking zinc. The cells contained catechol particles which

had separated from the solvent and had only just begun to coalesce.

These three cells (fig. 3) show plainly the import-

ant stages in the formation of the auto-complex coacervate in affected plant cells and emphasize that it starts with the well-known process of syneresis

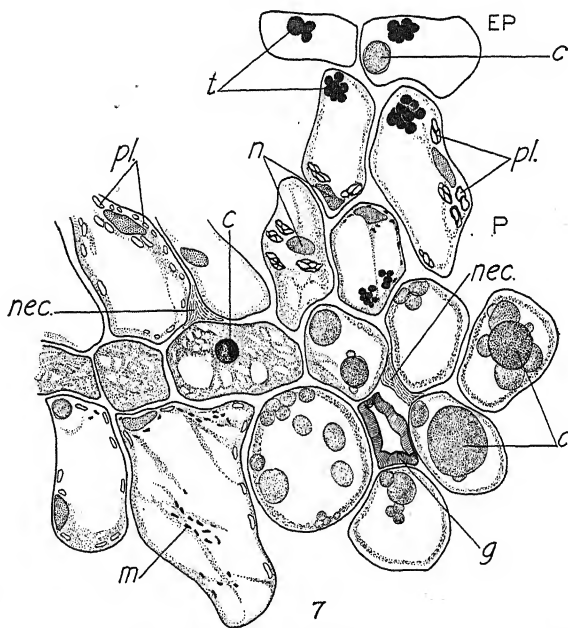
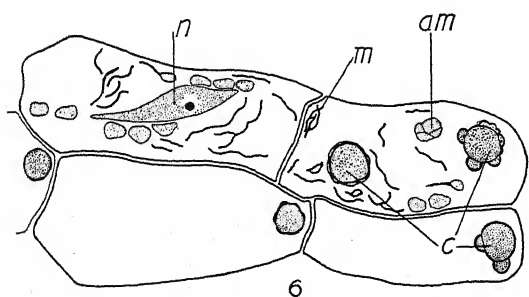
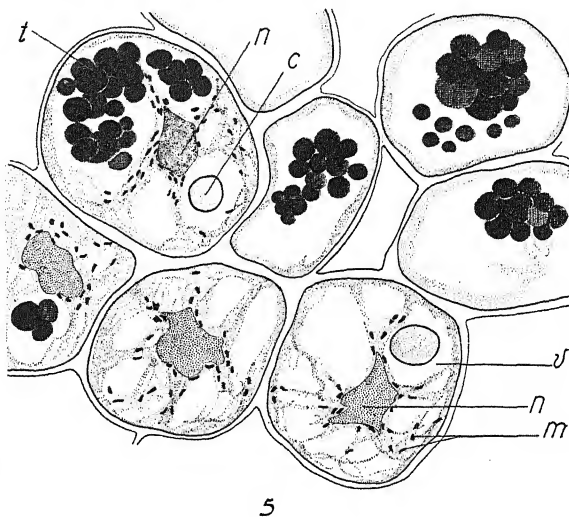
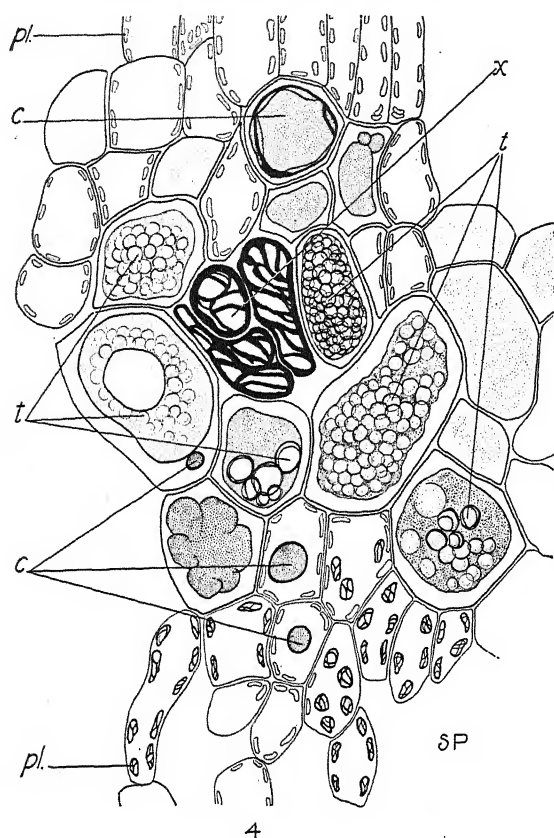


Fig. 4-7.—Fig. 4. Portion of a transverse section of an apricot leaf affected with zinc deficiency in which the perivascular cells were replete with masses of catechol. In the immediate vicinity of the bundle (x) the catechol masses formed a continuous phase enmeshing a number of vacuoles (t). Other cells contained spherical masses of catechol (c) enveloped by precipitation membranes and may be considered auto-complex coacervates. The plastids (pl) showed abnormalities.—Fig. 5. Cells from the apical region of a vegetative shoot of an affected apricot tree. The catechol material in some vacuoles (v) condensed into globular masses (t); in others it formed an aggregate (c) enveloped by a precipitation membrane. Nucleus (n); mitochondria (m).—Fig. 6. Coacervated material demonstrated by the molybdenic reagent in hypoplastic cells from root of an orange seedling which had grown seven months in a nutrient solution without zinc, (am) amyloplast; (c) coacervates exhibiting vacuolated envelopes of phospho-lipoid material; (m) mitochondria (frequently agglutinated); (n) elongated nucleus.—Fig. 7. Portion of a transverse section of a walnut leaflet, (*Juglans hindsii*), dwarfed and chlorotic by little-leaf. The hypertrophied palisade cells had a definite polarisation of contents, indicated by the position of vacuolar precipitates (t) at one, and nuclei (n) and plastids (pl) at the other pole. The perivascular cells contained globular masses (c) of catechol. Gum (g) and necrotic material (nec) occurred in intercellular spaces.

and ends with a globular mass of catechol surrounded by a layer of phospho-lipoid material floating in the vacuolar sap.

Apricot leaves taken from trees showing little-leaf (zinc deficiency) symptoms show striking derangements, especially in the later part of the growing season (Reed, 1938b). Samples taken during the period of rapid growth in the spring showed no serious derangements due to the lack of zinc. The nature of affected cells is shown by figure 4 which was drawn from a section of an affected leaf collected October 15, at Delhi, California. The material had been fixed in Nemec's solution and double stained with acid fuchsin and methyl green. The derangements were more evident in the perivascular and spongy parenchyma than in the palisade cells although in the latter the plastids were shrunken. There was hypertrophy of many cells, and in the spongy parenchyma (SP) the plastids were surcharged with starch due, apparently, to an inhibited cytase activity. The cells surrounding a xylem element (x) contained catechol material in several forms (t and c) which, as previously shown, is related to the little-leaf condition (cf. Reed, 1938b). The formation of the various aggregates followed some desolvating activity which involved separation and coalescence of catechol compounds. The large vacuolate masses (t) of catecholic material indicate that the shift of equilibrium was so abrupt that it allowed no time for complete separation and, as a result, some droplets of the solvating liquid were held within the aggregate. These masses seem similar in nearly every way to the "tannin vesicles" described by Massee (1895) in pathological cells of orchid leaves. The smaller globular masses (c) of catecholic material indicate a strong activity of catechol oxidase forming + charges on the surface which would attract the basic choline groups of phosphatides and form an enveloping lipoidic layer. Both of these components may be demonstrated with the molybdenic reagent by which the central mass of the coacervated catechol was colored light brown and the outer layer of phosphatide blue. The phosphatide layer of some coacervates like the upper (c) in figure 4 was vacuolated. When sections were double stained with acid fuchsin and methyl green, the core of catechol absorbed the basic methyl green.

We conclude from our investigation of fresh, as well as fixed materials, that the vacuolar solution contains both the oxidizable phenolic compounds and an agent (polyphenol oxidase) which is able to catalyze their oxidation. These phenolic compounds are, nevertheless, protected from oxidation in healthy cells by the presence of hydrogen donors such as ascorbic-dehydroascorbic or the dihydroxymaleic acid systems, even more by the presence of such thiol-containing groups as cystein and glutathione, the latter of which is able to act as a reservoir of hydrogen for the cell. This relationship can be used as the basis for explaining the seasonal variations in the amounts of these bodies previously discussed (Reed, 1938 a and b), since it is known that thiol

compounds and reductases are more abundant in rapidly growing than in mature organs. During the early part of the growing season the leaf cells would have more hydrogen donors and would, therefore, keep the catechol particles randomly distributed throughout the cell vacuole. When growth inhibiting influences such as senescence or nutrient deficiencies intervened, destroying the equilibrium between oxido-reductant factors, the desolvating action already mentioned would produce vacuolar aggregates of the sorts now under discussion (fig. 4).

Skoog (1940) has demonstrated the destructive influence of the oxidases on the growth auxin in plants deprived of zinc, finding that the tissues of such plants exhibited an enhanced oxidative capacity and a corresponding decrease in the growth auxin. It is evident from his work that the abnormally high oxidative capacity of the zinc-deficient plants was responsible for the rapid rate of auxin inactivation. Furthermore, he discovered a phenomenon which has an important bearing on the zinc-catechol-oxidase relationship, namely, an enhanced oxidation of catechol in the extracts of zinc-deficient plants. The abundance of brown compounds often obscured the benzidine test for oxidase.

Another separation of phases was mentioned (Reed, 1938 b) in the lipid-protein complex of the plastids of leaves from severely affected apricot trees after growth had ceased. Droplets of lipid material and vacuolization of the stroma were early stages in the derangements of structure and function which ended in the disappearance of the plastids.

We can now present confirmatory evidence of the rôle of catechol and related phospho-lipoids in the formation of coacervates from a study of cells from the sub-apical region of affected apricot shoots. The effect of the deranged metabolism is clearly seen in the hypertrophy of post-meristematic cells which contained vacuolar aggregates of phenolic materials. Figure 5 shows cells from an affected apricot shoot collected January 24, when the tree was in a state of comparative dormancy. The section had been fixed in Nemec's solution and double stained with acid fuchsin and methyl green. Each vacuole was outlined by pink cytoplasmic strands along which mitochondria (m) showed as many red rods. The catechol in some of the vacuoles formed small globular aggregates (t) which the bichromate of the killing fluid stained yellow. In others the catechol had been coacervated into spherical masses (c), surrounded by precipitation membranes which were darkly stained by virtue of their ability to absorb both acid fuchsin and methyl green from the staining mixture. The cells show the comparative independence of the individual vacuoles and exhibit nuclei compressed into a star shape by the pressure of the adjacent vacuoles.

Further evidence of the two phases in auto-complex coacervates will be seen in figure 6 showing cells of an orange root which had grown in a zinc-deficient culture solution. The section from which

the drawing was made had been treated with the molybdenic reagent and, as a result, showed plainly the vacuolate phospho-lipoid layer surrounding the globules of catechol. The enveloping layer was blue and the central globule light brown after treatment with molybdenic reagent. The brown color of the central globule was due, undoubtedly, to the oxidation of the catechol to a diketone by the reagent.

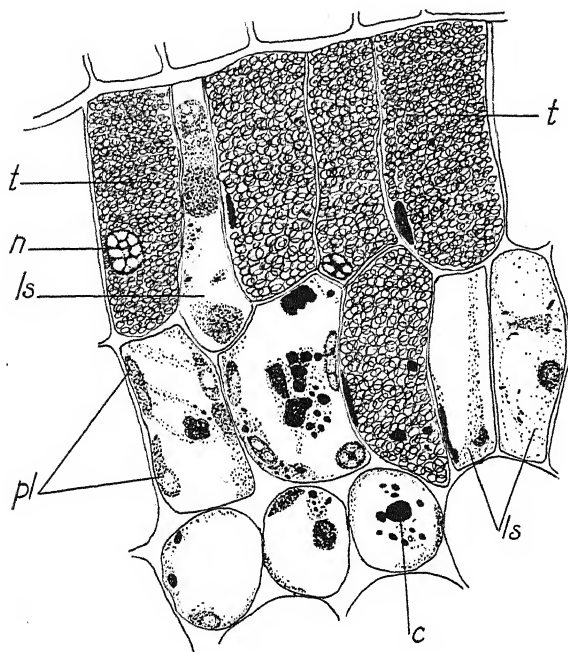


Fig. 8. Portion of a leaflet of *Juglans regia* affected with little-leaf. The hypertrophied palisade cells were either filled with catechol masses which formed a continuous phase (t), or exhibited varying stages of lysis (ls). The spongy parenchyma cells often contained globular aggregates (c) of catechol. The nuclei (n) were often indistinct.

The idea of two distinct phases in those globules is supported by Caruso's (1939) successful microphotographs of similar inclusions in cells of *Cephalocereus*.

The condition of the cells and the phenolic materials in leaves of the walnut affords important information because in them the polyphenol oxidase is very active. Both the cultivated walnut (*Juglans regia*) and the native (*J. hindsii*) were studied. The affected leaves, which were extremely dwarfed and chlorotic as a result of zinc deficiency, were collected at Delhi, California, on August 12 and fixed in Nemec's killing fluid. These leaves, which are naturally rich in phenolic materials, afford some interesting comparisons with those previously discussed.

The perivascular cells, like those of the peach leaf (Reed, 1938), contained large quantities of phenolic materials and appeared to be severely affected.

The palisade cells of the affected walnut leaves (fig. 7) were hypertrophic, hypoplastic and had a strong tendency to polarization of the contents,

similar to the condition we described in 1935 in affected orange leaves and in 1938 in affected avocado leaves. These conditions were never observed in healthy walnut leaves. The nuclei and plastids of affected cells were at one pole, and vacuolar precipitates were grouped at the opposite pole. Their plastids were small and probably unable to function efficiently. Mitochondria were present but seemed to be in an arrested stage of development. Many of the cells had suffered such extreme lysis that they were practically empty. Necrotic material, which had a strong affinity for hematoxylin, was frequently seen in intercellular spaces (fig. 7, nec), as well as gum.

Catechols were abundant in most cells, occurring either as large masses filling the vacuoles (fig. 8) or as golden brown globules (fig. 7). The conditions resembled the second stage of syneresis shown in figure 3. Globules of catechol which could be regarded as auto-complex coacervates were not readily detected by the staining techniques used. The molybdenic reagent failed to demonstrate any phospho-lipoid materials in association with the catechol globules in the walnut leaf cells. The absence of a desolvant agent like phospho-lipoid would account for a distribution of catechols like that represented in figures 7 and 8, especially in the presence of an active polyphenol oxidase. The absence of phospho-lipoid could result in the diffusion of catechol, sometimes seen in severely affected leaves. Their poverty in other lipoids is reflected in the absence of chlorophyll.

Where both kinds of aggregates occur, as in affected apricot leaves, there may not be enough phospho-lipoid or other desolvant like lecithin to form an auto-complex coacervate of all the material. Klercker also found a precisely similar state of affairs in another tree which is rich in tannin, namely, *Quercus pedunculata*.

The cells in figure 8 afford some interesting contrasts. Cells whose vacuoles were almost completely filled with vacuolated aggregates were adjacent to cells whose vacuoles contained a few globular masses of catechol, leaving the vacuole quite free of other inclusions. From this and other observations one may conclude that proteolytic and oxidative agents sometimes work simultaneously in affected plant cells, but not always.

SUMMARY

Knowledge of the cellular inclusions which seem characteristic of many diseased cells has been advanced by means of new developments in technique which are outlined in the present paper.

These spherical inclusions in the cell vacuoles of zinc deficient plants are formed by a process in which some of the colloidal material in the vacuolar solution of the cell becomes condensed into a spherical mass instead of being distributed at random in the solvent. These aggregates may be formed by a process of coacervation, which may be to a certain extent considered a separation of phases, but they have a boundary layer which is more than the theo-

retical interphase. It is a precipitation membrane composed of oriented molecules.

In the cases we have studied, the mass of the aggregates appears to be made up of catechol. The precipitation membrane or bounding layer is a phospholipoid, identifiable by suitable chemical reagents.

Coacervated aggregates have been found in the vacuoles of hypoplastic cells of leaves and in the post-meristematic cells of growing shoots of apricot trees suffering from little-leaf (zinc deficiency), as well as in the cells of leaves of walnut trees affected with the same malady. Evidence of a pathological

condition in the walnut leaves was also indicated by the escape of necrotic material from the cells and its accumulation in intercellular spaces, coupled with the formation of gum in intercellular spaces adjoining severely affected cells.

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HORMODENDRUM RESINAE (LINDAU), AN INHABITANT OF WOOD IMPREGNATED WITH CREOSOTE AND COAL TAR ¹

C. M. Christensen, F. H. Kaufert, H. Schmitz and J. L. Allison

COAL TAR products, especially creosote, have been used for more than a hundred years to protect wood from attack by fungi, insects, and other organisms, and the best evidence of their preservative value is the long service given by wood impregnated with them. Some of the compounds contained in these preservatives are very toxic to most fungi, and since each cubic foot of wood impregnated with them contains 8 to 20 pounds, or 1 to 2½ gallons, of preservative, such wood scarcely would be suspected of har-

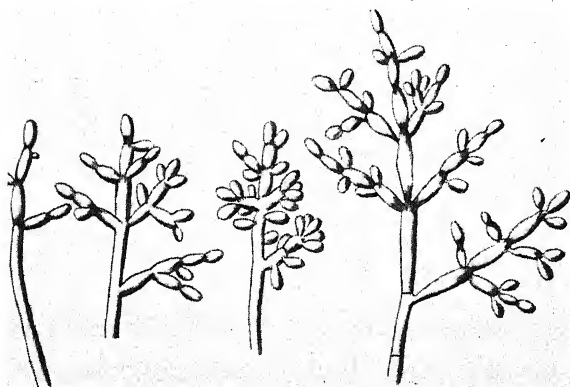


Fig. 1. Young sporophores of *H. resinae*. The three at the left are 48 hours old, the larger one at the right 72 hours old. $\times 900$.

boring living organisms. A brown mold has been observed occasionally by us and others² on small pieces of impregnated wood (usually from ties or poles that had been in service for some time) placed in moist chambers or on agar during routine tests of such wood for resistance to decay. Because of the known toxicity of these preservatives to microorganisms in general, this sporadic appearance of a fungus on treated wood was more or less disregarded as a laboratory phenomenon. Our present investigations prove that this mold is a common, if not an almost universal, inhabitant of wood treated with coal tar products, including creosote.

In the fall of 1939, several tie plugs overgrown with this brown mold were received from Dr. Herman von Schrenk of St. Louis. These plugs had been impregnated with coal tar early in the summer of 1939, packed tightly in square bundles, and stored in a shed where the humidity was high. The general appearance of the fungus on the tie plugs is shown in figure 2A.

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² Dr. C. A. Richards, Forest Products Laboratory, Madison, Wisconsin, and J. D. Burns, Page and Hill Company, Robbinsdale, Minnesota.

IDENTITY AND DESCRIPTION OF THE FUNGUS.—Several pure cultures of the fungus were made, and one was sent to Dr. Johanna Westerdijk, director of the Centraalbureau voor Schimmeldkulture, Baarn, Holland, for identification. The culture was there compared with the available species of *Cladosporium* and *Hormodendrum*, and was found to differ from them, the opinion being expressed that it might be an unknown species of *Cladosporium* (*Hormodendron* or *Hormodendrum*).

Cultures of the fungus on the usual artificial media, such as malt and potato dextrose agars, are white when very young, but within a few days become dark olive brown or nearly black, due to a dark pigment in the walls of the hyphae. The mycelium is appressed, but the great number of spores give the cultures a powdery appearance. Numerous black, wrinkled sclerotia from 0.1 to 2 mm. in diameter are formed on the surface of and within the agar of six-week- to two-month-old cultures.

Sporophores and spores are produced in cultures twenty-four hours old, typical ones being illustrated in figure 1. As these become older, the spore masses form rather dense heads, varying from spherical to conical or cylindrical, at the tips of the sporophores. Each head contains hundreds of conidia.

The spores are pale olive brown and vary in shape from spherical to elongate oval. Cultures from the tie plugs were grown on balsam fir wood, filter paper, leaves, and prunes; and spores produced by the fungus on these media were mounted in a drop of cool agar and measured with the aid of an ocular screw micrometer and an oil immersion lens. The average length of 100 spores was 4.5 microns, the range being 3.2 to 9 microns. The average length of the first 50 measured differed only 0.5 micron from that of the second 50. One hundred spores from a culture isolated from a butt-treated western red cedar pole in Wilmington, Delaware, were similarly measured and averaged 5.1 microns in length. The length of spores from other cultures of the fungus obtained from other areas and grown on several different media did not differ significantly from the above averages.

The fungus undoubtedly is a species of *Hormodendrum* or *Cladosporium*. A few words concerning the genera in question may be desirable. Lindau, in Rabenhorst's *Kryptogamen Flora*, Vol. 1, Pt. 8, and also in Engler and Prantl's *Die Natürlichen Pflanzenfamilien*, and Saccardo in *Sylloge Fungorum*, merely repeat Bonorden's original description of *Hormodendrum*, made in 1851, which is: "Sterile hyphae procumbent, branched, septate. Conidiophores upright, septate, brown, repeatedly branched or only a little branched. Conidial chains acrogenous, often comprising the entire branch down to the stem. Conidia spherical or egg shaped, olive green or brown, one celled." The original description of *Cladosporium* is almost identical, differing chiefly

in that *Cladosporium* was said to have two- or several-cell spores.

That portion of Die Natürlichen Pflanzenfamilien dealing with these two genera was written by Lindau in 1889 (according to his statement in Thesaurus Litorarum Mycologicae, vol. 1, p. 862). The similar portion in Rabenhorst's Kryptogamen Flora was written by Lindau in 1906. In the earlier work he states that the spores of *Hormodendrum* have one cell, and those of *Cladosporium* two cells. In the later work he is less positive of this distinction, and to his description of *Cladosporium* he appends the remark that the genus is exceedingly variable and difficult to describe, and that he would prefer to consider the multicelled nature of the conidia, and the ability of the conidia to bud as characteristic of *Cladosporium*. In spite of this, three of the 63 species of *Cladosporium* he describes in Rabenhorst's Kryptogamen Flora are said to produce one-celled spores only.

Among the species of *Hormodendrum* that Lindau describes in the Kryptogamen Flora is a new one, *H. resiniae* Lindau, found growing on resin of *Picea excelsa* in the Sachsenwald near Hamburg. In the short description of this species he states: "The conidia are in short chains, which are either sparsely distributed along the stem of the main conidiophore or united in heads. Conidia ellipsoid-spindle shaped, bluntly pointed at both ends, greenish brown, transparent, 5.5 to 7 microns long, 3.5 to 4.5 microns thick." He further states that the conidiophores show the typical *Hormodendrum* form, somewhat as in *H. hordei*, an illustration of which is given. Of all the species of *Hormodendrum* and *Cladosporium* he describes, the above *H. resiniae* most closely agrees with our fungus. Its growth on resin may be significant also, as will be shown later.

Since Lindau's time, species seemingly intermediate between *Hormodendrum* and *Cladosporium* have been described, and it has been suggested that the two genera be united. Our fungus certainly does not form two-celled spores, even in cultures a year or more old; if this be a legitimate generic character, we are justified in considering it a species of *Hormodendrum*. Since it conforms rather closely to the description of *H. resiniae* Lindau, we have tentatively referred it to this species.

OCCURRENCE OF *H. RESINAE* IN NATURE.—Earlier observations by the writers that *H. resiniae* was present in coal tar and creosote-treated wood obtained from a number of localities, stimulated a more detailed study of its occurrence in treated wood and its general distribution in nature.

Isolations from wood impregnated with coal tars and creosotes.—Most of the creosoted wood sampled in this study was from poles or ties, these products making up a large part of the material treated with creosote and coal tar. Samples were taken from creosoted telephone poles and railroad ties in service, or recently removed from service. The fungus was isolated and grown on malt agar and malt agar containing 0.24 to 1 per cent coal tar creosote (the latter hereafter referred to as agar-creosote medium).

Such concentrations of creosote in malt agar prevent the growth of most of the common contaminants but permit the growth of *H. resiniae*. That *H. resiniae* can grow on malt agar containing higher concentrations of creosote will be shown later.

Samples were taken near the ground line from twenty-nine creosoted poles in Minnesota, Wisconsin, Pennsylvania, and Delaware; and *H. resiniae*

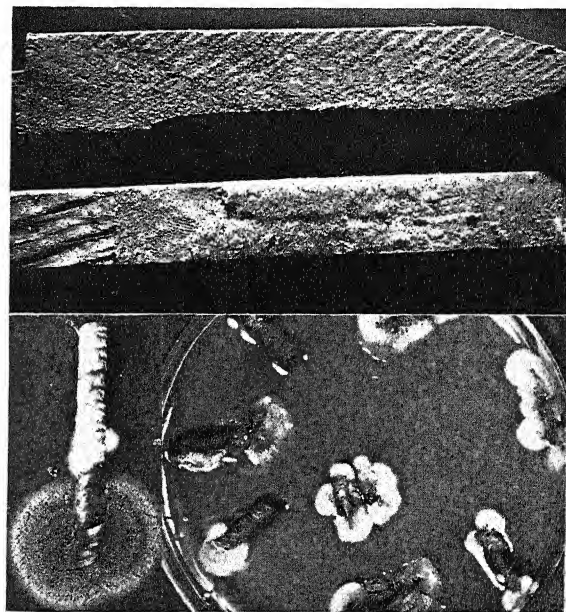


Fig. 2.—A (above). Tie plugs impregnated with coal tar. The lower one is overgrown with *H. resiniae*.—B (lower right). Two increment borings from a pressure treated southern pine pole cultured on agar-creosote medium. *H. resiniae* was obtained from all four pieces of each boring.—C (lower left). An increment boring from a butt treated western red cedar pole, cultured on agar-creosote medium. *H. resiniae* has grown out of only the dark, creosoted portion.

was isolated from twenty-seven of them. It was likewise isolated from six of the eight creosoted ties sampled in Minnesota, and from all of the eleven ties from Ohio, from all of the four ties sampled from Louisiana, three from the State of Washington, and from ties sampled from Missouri and Indiana. The fungus was found in both butt-treated cedar poles and full-length pressure-impregnated southern pine poles. The ties from which it was isolated include such species as jack pine, southern pine, red oak, birch, and red gum. Other creosoted material from which the fungus was isolated includes: a Douglas fir cross arm impregnated with creosote and stored at the treating plant for three months, southern pine fence posts pressure treated with creosote and stored in a basement room for several years, several creosoted southern pine fence posts in service for seventeen years in Iowa, and the tie plugs referred to above.

Service records were available for most of the materials sampled. These records revealed that *H. resinae* was obtained from almost every creosoted pole in service for two years or more. The recovery of *H. resinae* from the cross arm and from posts that never had been in service may be accounted for by their storage out-of-doors or in a room sufficiently humid to permit the fungus to grow in the treated wood. The fact that samples of freshly creosoted wood, placed in a humid chamber and inoculated with a spore suspension of *H. resinae*, will be overgrown rapidly by the fungus is added evidence that length of service has less bearing on the prevalence of the fungus than such factors as moisture and presence of inoculum.

The samples were taken from telephone poles by means of an increment borer, each boring being 2 to 3 inches long. In the case of western red cedar, which is impregnated by steeping the lower portion of the pole in an open tank of alternately hot and cold creosote, the boring extended through the creosoted wood and included an inch or more of nontreated wood. Most of the latter borings were cultured by breaking them into several pieces, the pieces then being placed on agar in a Petri dish or each piece being put on agar in a separate test tube. *H. resinae* grew out of almost every portion of each boring (fig. 2B), indicating that it extended deep into the wood and was not merely on the surface. When the isolations were made on malt agar containing no creosote, usually only *H. resinae* grew out of the creosoted wood and no organisms grew from the inner, nontreated portion. In a few cases an as yet unidentified fungus grew from the inner, nontreated portion.

It would of course be theoretically possible for spores of *H. resinae* to be carried in from the surface of the pole by the increment borer when the sample was taken. Were this the case, however, one would almost certainly obtain it from the noncreosoted inner portion of the boring. Therefore, it seems fairly certain that the fungus grows in as far as the creosote extends, and no farther. Other organisms sometimes are present in the nontreated inner portion of the wood and might prevent the growth of *H. resinae*. The inability of *H. resinae* to compete with common molds on nontreated wood will be discussed below.

Most of the creosoted ties were sampled by chopping from each a block about six inches long and extending in to the center of the tie. The surfaces of each block were flamed, then the block was split lengthwise to expose a surface extending from the outside of the tie into the center. Usually the creosote did not extend more than two inches in from the outside. Small pieces of wood were taken adjacent to one another across both the creosoted and noncreosoted areas. In almost every case *H. resinae* grew out from every piece taken from the creosoted portion but from few or none of those taken from the noncreosoted portion. Most of these isolations from ties were made on malt agar to which no creosote had

been added, but in only a very few cases did any organism other than *H. resinae* grow out of either the creosoted or noncreosoted wood. This is additional evidence that the fungus is a regular inhabitant of creosoted wood but does not grow readily on noncreosoted wood in nature. Further evidence will be presented that the fungus not only tolerates coal tar or creosote but may derive nourishment from it.

The results of these studies indicate that *H. resinae* must be a very common and general inhabitant of wood impregnated with coal tar and coal tar creosote throughout much of the United States, and is not limited to one product or one region.

Possible natural habitat and sources of inoculum.—It seems at least probable that any organism, such as *H. resinae*, which grows in wood impregnated with coal tar or creosote, which are man-made products of relatively recent origin, must have been on the scene long before these products appeared; therefore, it was of some interest to look for a natural source of inoculum.

Creosoted wood blocks, and agar to which sufficient creosote had been added to prevent the growth of most organisms except *H. resinae*, were exposed repeatedly in the laboratory and out-of-doors for as much as a week at a time, and then incubated for weeks or months in the laboratory, but not a single culture of *H. resinae* was obtained. While this at least indicates that few spores were present in the air at the time and place of the exposures, it does not exclude the possibility of the spores being predominantly air borne and being present in abundance during certain times and at certain places.

Plant refuse was placed on creosote-agar in Petri dishes and incubated without obtaining the fungus. Numerous isolations from greenhouse soil on the same agar-creosote medium yielded only one culture of *H. resinae*. Soil samples were taken from nine different locations within an eight-mile radius of the University Farm, St. Paul, Minnesota, including cultivated and uncultivated land, one of them from within a few yards of a large creosoting plant, and cultured on agar-creosote medium. *H. resinae* was not obtained from any of these.

Samples of asphalt street pavement were taken from five different places and cultured on agar-creosote medium and also on creosoted wood blocks. Three of these yielded *H. resinae*, but in no great abundance. In all others the asphalt was slowly overgrown by a fungus, as yet unidentified, mentioned above as being isolated from a few samples of creosoted wood. This same organism grew out from every part of almost every soil sample on the agar-creosote medium, although it grew but poorly on this medium.

Resinous bark and twigs from fifteen blue spruce trees affected with *Cytospora* canker were cultured on agar-creosote medium and also on creosoted wood blocks. *H. resinae* grew out from thirteen of these, and typically grew out of the entire bark surface, not only from isolated spots. It is possible that this and other resinous woods form one of the natural

habitats of *H. resinae*, but the evidence at present is not sufficient to make this more than a possibility.

Creosoted wood blocks in jars that were opened frequently, and plates of agar-creosote medium, were regularly maintained as controls in all these isolation experiments, but in no case has *H. resinae* or any other organism appeared on them.

THE BIOLOGY OF *H. RESINAE*.—The studies described below were made to determine some of the factors influencing the growth of *H. resinae*, its tolerance to coal tar products, and whether it might be able to utilize certain coal tar products.

Effect of temperature.—Petri dishes containing 5 per cent malt agar were inoculated with a spore suspension of *H. resinae*, kept at room temperature (20 to 25°C.) for three days, then placed in incubators maintained at 5, 10, 15, 20, 25, 30, and 40°C. Ten days later the colonies at 5° were 0.5 cm., those at 30° were 3.77 cm. and those at 40° were 1.37 cm. in diameter. Compared with fungi in general *H. resinae* has a rather high optimum and a wide temperature range. Neither the minimum nor maximum temperatures were reached in this experiment.

Effect of pH.—Yield of the fungus, as measured by weight of mycelial mats, from triplicate flasks of Pope's medium adjusted to twelve different pH concentrations from 3.0 to 10.5 varied so much as to preclude a definite statement as to minimum, maximum, and optimum pH concentration for growth. The weight of mycelium produced in fourteen days was almost the same at pH 3 as at pH 8, lower at pH 9.6 and higher from 3.5 to 8.0. At the end of the experiment, the media at all but the lower and two upper pH concentrations had a pH of 7.0. Like many other microorganisms, *H. resinae* appears to grow over a rather wide pH range, and has the ability to alter the original pH of the medium.

Effect of inorganic toxicants.—The resistance of *H. resinae* to CuSO_4 , ZnCl_2 , and AsO_3 , respectively, was determined by adding these compounds to malt solution (25 grams of malt extract in 1,000 cc. of water), in various concentrations. The culture flasks

were inoculated with spore suspensions of the fungus. Approximately 150 parts of copper sulphate and 380 parts of zinc chloride per million of malt agar were required to inhibit growth. Wood destroying fungi, such as *Lenzites trabea*, Madison 517, *Trametes serialis*, and *Lentinus lepideus*, have a far higher tolerance to these common inorganic toxicants, and the killing concentrations as given by Bateman (1937) are many times as high as those totally inhibiting the growth of *H. resinae*. In case of AsO_3 , 1,200 parts of arsenic trioxide per million did not decrease the growth of the fungus much over that of the controls. It would appear from these results that *H. resinae* is fairly resistant to arsenic. However, there are many fungi tolerant to high concentrations of arsenic, among them *Lenzites trabea* and a number of common molds, Bateman (1937) and Kaufert and Schmitz (1937).

It would appear from these results, that *H. resinae* is not an unusual organism in its resistance to some of the common inorganic toxicants used as wood preservatives. Judging from these results, one would not expect to isolate *H. resinae* from wood treated with these inorganics more often than some of the other organisms given above.

Effect of coal tar products.—The tolerance of *H. resinae* to coal tar and creosote was determined by the standard agar-plate method, and by treating wood, filter paper, and glass beads or slides with creosote and coal tar and inoculating the treated materials with spore suspensions of the fungus.

Agar tests.—These tests were made by the procedure described by Schmitz, *et al.* (1930). Characteristics of the creosotes and coal tars used in these studies are shown in table 1.

These creosotes and coal tars are representative samples of materials used by the wood preserving industry, at the time the samples were obtained. The results of toxicity tests with creosotes A and B are shown in table 2.

A further test with creosote C and coal tars A and B, in which the agar-creosote or agar-coal tar mix-

TABLE 1. Characteristics of creosotes and coal tars used in tests with *H. resinae*.

	Creosote			Coal tar	
	A	B	C	A	B
Specific gravity at 38°C./15.5°C.....	1.067	1.044	1.074	1.173	1.167
	Per cent	Per cent	Per cent	Per cent	Per cent
Insoluble in benzol.....	0.02	0.10	0.28	7.05	5.54
Water	0.1	Trace	0.60	Trace	0.80
Coke residue	0.9	1.66	1.38	... ^a	... ^a
Tar acids ^a	... ^a	1.80	2.2	2.5
Fractional range:					
Up to 210°C.	2.3	3.1	1.3	1.5	1.9
210-235	10.4	27.5	11.7	5.2	4.7
235-270	24.3	23.6	26.6	8.9	8.2
270-315	15.9	14.6	17.9	8.8	9.1
315-355	18.2	18.7	18.0	12.2	11.9
Residue	28.9	12.5	24.1	63.3	63.9

^a No values available.

TABLE 2. Growth of *H. resinae* on malt agar containing creosote.

Conc. of creosote in agar	Diameter of colony in centimeters after 23 days	
	Creosote A	Creosote B
0.5	4.0	3.0
0.6	3.0	3.0
0.7	2.5	2.8
0.8	2.5	2.4
0.9	2.0	2.0
1.0	2.0	1.9
2.0	1.8	1.8
3.0	1.6	1.0
4.0	1.4	0.5
5.0	1.2	No growth

tures were placed in jars with loosely fitting screw caps and were inoculated with spore suspensions of *H. resinae* every seven days for three months, revealed that the organism would grow on agar containing as much as 10.0 per cent creosote or coal tar. The conditions in these later tests were very different from those in the tests summarized in table 2 because there was opportunity for loss of some of the lower boiling fractions, and presence of viable inoculum was assured by repeated inoculations.

The extreme tolerance of *H. resinae* to coal tar products is best illustrated by comparing the results reported above with the results obtained in parallel tests with creosotes A and B and several common wood-destroying fungi. Both creosotes killed *Coniophora cerebella* at a concentration of 0.05 per cent, *Madison 517* at a concentration of 0.3 per cent, *Lenzites sepiaria* at 0.3 per cent, and *Trametes serialis* at 0.2 per cent.

Wood tests.—Aspen blocks 1 by 1 by 1½ inch were impregnated with creosote C and coal tars A and B (see table 1 for characteristics of these oils) by first subjecting the blocks to vacuum treatment and then adding the warmed coal tar and creosote without breaking the vacuum. Retentions in the blocks varied from five to twenty pounds per cubic foot. After impregnation, one-half the blocks treated with each of the above oils were heated to 100°C. for eighteen hours. This treatment was considered sufficient to remove most of the volatile components of these oils. Twenty blocks comprised each set. Each set of twenty blocks was put in a separate humid chamber and atomized with spores of *H. resinae* suspended in sterile distilled water.

Mycelial wefts became visible on some of the blocks three days after inoculation, and three months after inoculation all the blocks treated with coal tar were covered with abundantly sporulating mycelium. Mycelium and spores were less abundant on the blocks treated with creosote but were sufficient to give the blocks a definite moldy appearance.

Blocks cut from recently creosoted Douglas fir cross arms, cedar poles, and southern pine posts and placed in humid chambers soon became covered with mycelium of *H. resinae*. It was not necessary to

inoculate these samples of creosoted wood cut from material that had been treated for some time, the surface of the material apparently having sufficient inoculum from natural contamination from the air or other sources.

The growth of *H. resinae* on wood freshly treated with creosote or coal tar and its inhibition in agar plate tests by 5.0 and 10.0 per cent concentrations of the same materials, emphasizes again the great differences between agar and wood as test media.

Filter paper tests.—Cellulose filter papers were sterilized, one edge dipped in samples of coal tars A and B and creosote C, placed in jars and atomized with a spore suspension of *H. resinae*. The fungus grew and fruited readily and abundantly on the portion of the filter paper soaked in tar, less readily and less abundantly on that dipped in creosote, and sparsely or in patches, or in many cases not at all on the filter paper alone.

Glass slide and bead tests.—Clean glass slides, and glass beads cleaned with concentrated sulphuric acid, washed thoroughly with distilled water, and heated to a red heat to remove all organic matter, were dipped in samples of coal tar A and B and creosote C respectively, atomized with a spore suspension of *H. resinae*, and kept in tightly closed bottles. The spores germinated in both tar and creosote, grew and fruited fairly well on the tar and grew and fruited very sparsely on the creosote. On both, the growth was so sparse that microscopic examination was necessary to find it.

The results of these experiments indicate that *H. resinae* derives some nourishment from the constituents of typical coal tars and creosotes used as wood preservatives. The consistently better growth and sporulation on coal tar as compared with creosote, whether on glass beads, impregnated filter paper, or impregnated wood, obviously suggest that either coal tar contains more substances which the fungus can utilize as food or the creosote contains more compounds toxic to it. Very probably it is partly both. Experiments now are under way to determine this.

Ability of *H. resinae* to compete with other organisms on wood and in soil.—The common occurrence of *H. resinae* in creosoted wood in service, and our failure to obtain it from untreated wood, soil, and other materials commonly inhabited by saprophytic fungi, suggested that possibly it could not compete in ordinary media with common molds and bacteria, and grew in creosoted wood not only because of its high tolerance to coal tar products but because competition with other organisms did not exist in this medium. The ability of *H. resinae* to compete with other fungi on untreated wood was studied.

Strips of sapwood 1 by 2 by 16 inches were cut from a fresh red pine log, and one-half of these strips were sterilized and placed in screw-capped jars, the other half placed in jars without being sterilized. One-half of each series were inoculated with a spore suspension of *H. resinae* alone and the other half inoculated with a mixture of *H. resinae* plus *Peni-*

cillium, *Aspergillus*, and *Trichoderma*. The wood strips were examined at intervals of two weeks, for a period of six weeks, the jars being opened during each examination. The results were:

A. Wood sterilized and inoculated with *H. resiniae* alone. At the first examination the wood was totally covered with a heavy growth of *H. resiniae*; at the second examination other molds were starting to come in and at the end of the test *H. resiniae* was no longer visible. A species of *Penicillium* covered most of the surface of the blocks at the end of test and probably came in when the jars were opened for the first and second examinations.

B. Wood not sterilized, inoculated with *H. resiniae* alone. A trace of *H. resiniae* was visible on the lower ends of the pieces at the first examination but this rapidly disappeared, and at the end of the test the wood was uniformly covered with *Penicillium* and *Aspergillus*.

C. Wood sterilized and inoculated with *H. resiniae* plus other molds. At the first examination a trace of *H. resiniae* was present but as in B this rapidly disappeared and other molds predominated.

D. Wood not sterilized, and inoculated with *H. resiniae* plus other molds. No *H. resiniae* was found at the first examination, the surface of the wood being uniformly covered with the other molds used.

To summarize, then, *H. resiniae* grew luxuriantly on wood that had been sterilized to eliminate all competitive fungi, but it could not compete with the other fungi used in this experiment, and even after having become established was quickly overgrown by these other molds. After the third examination, the blocks were taken out of the jars and stored outside where the temperatures were below freezing most of the time. About a month later eight of the blocks were selected, spores washed from the surface with distilled water, and the spore suspensions cultured on agar-creosote medium. A few colonies of *H. resiniae* were obtained on several of the dishes, indicating that the organism still survived but that viable spores were not present in any abundance.

To determine whether *H. resiniae* could compete with other organisms in the soil, the following simple tests were made:

Approximately 100 cc. greenhouse soil were put in ten 250 cc. Erlenmeyer flasks, and five of them were kept for one hour in an autoclave at twenty lbs. steam pressure. Three of each series of five flasks were inoculated in a small area at one side with two drops of spore suspension of *H. resiniae*, and two of each were inoculated over the entire surface with a similar spore suspension, to determine if the fungus could survive and grow in sterilized and unsterilized soil. The flasks were kept at room temperature in the laboratory.

After three weeks isolations were made from three places in each flask of non-sterile soil which had been inoculated at one side: (1) opposite the point of inoculation; (2) in the center of the flask; (3) at the point inoculation. In each case from $\frac{1}{10}$ to $\frac{1}{3}$

cc. of soil was scattered over the surface of a Petri plate containing agar-creosote medium. *H. resiniae* was isolated in only one out of the nine attempts, and in that case from the point of inoculation. It was isolated from both of the samples inoculated all over, but only a few colonies appeared. It grew visibly on the surface of the sterilized soil in all cases and was reisolated from every sample.

H. resiniae was, however, isolated from each of five samples of soil from near the base of five different creosoted poles, but one would expect a fairly heavy concentration of spores there. It was also isolated from soil and duff beneath young tamarack trees in an arboretum and from soil beneath a dead spruce tree, the bark of which was covered with exuded resin. It seems unlikely that *H. resiniae* is a common soil inhabitant; at least the present evidence indicates that it is unable to survive long in competition with the multitude of organisms in some soils, and in those few instances when it was isolated from the soil it is likely that the soil was contaminated by spores from nearby creosoted or resinous wood.

Effect of H. resiniae on wood.—The fact that *H. resiniae* was found so abundantly in wood impregnated with coal tar and creosote suggested that it might have some effect on the strength of the wood. That this effect probably would be small was indicated by the excellent condition of all of the creosoted wood samples from which the organism was isolated. These observations and the resistance of creosoted wood to mechanical wear even after 30 to 40 years of service are sufficient proof that *H. resiniae* could not appreciably affect the strength of such wood.

To obtain some information on this point, small sapwood blocks of longleaf pine, shortleaf pine, Ponderosa pine, soft maple, black gum, paper birch, and aspen were inoculated with this fungus and incubated for ten months. Most of these woods are known to be very susceptible to the action of wood destroying fungi, but in no case was there any evidence that *H. resiniae* was capable of causing loss in weight or other visible changes in the physical properties or appearance of the test blocks, other than a slight staining of the surface. In all cases the fungus grew over the surface of the test blocks and spores were produced in profusion.

Further tests are necessary to determine accurately the effects of this fungus on wood, because loss in weight, though good indication of loss in strength for wood attacked by wood-rotting fungi, is less strikingly correlated with loss in strength where other wood inhabiting fungi are concerned. Chapman and Scheffer (1940), working with blue staining fungi in southern pine wood, found that very small specific gravity changes were often accompanied by large changes in strength, particularly in toughness, which was greatly reduced by many fungi causing blue stain.

Associated with other organisms in creosoted wood.—When the agar-creosote medium was ex-

posed to air in an attempt to isolate *H. resinae*, occasional colonies of a bacterium were obtained. This same bacterium was isolated in considerable abundance on agar-creosote medium from plant refuse and from all soil samples. In isolations from soil, some plates were entirely overgrown with this bacterium for some time before *H. resinae* appeared. It also was isolated from many of the samples of creosoted wood from poles and ties and appears to be almost as common an inhabitant of such material as *H. resinae*. Like the white, as yet unidentified, fungus previously mentioned, the bacterium was often obtained from deep in the wood. It also was isolated from all asphalt samples cultured. It seems doubtful if it has any intimate biological connection with *H. resinae*, but its significance, if any, is not yet known. It is mentioned as another organism more or less regularly inhabiting creosoted wood.

DISCUSSION.—Although wood impregnated with coal tar or creosote is highly resistant to the attack of insects and organisms that deteriorate untreated wood, it is by no means free from microorganisms. That it is a fairly favorable habitat for the growth of *H. resinae* and an unidentified bacterium appears evident. The fact that the organisms here reported grow commonly in such treated products is somewhat surprising but certainly tends in no way to reflect on the proven value of creosote as a wood preservative. There is no evidence that *H. resinae* or the unidentified bacterium tend in any way to reduce the preservative value of these compounds, nor does it seem likely that they affect any of the intrinsic properties of the wood that they inhabit. They are, so to speak, casual interlopers who find among the multitudinous compounds contained in creosote or coal tar some materials that they can utilize; and, in addition, they must possess an unusually high resistance to the numerous highly toxic compounds that they cannot utilize. It seems unlikely that they "prefer" such a medium for growth, if one may use so teleologic a term, but that they do grow commonly in creosoted wood scarcely can be doubted in light of evidence here reported.

SUMMARY

A fungus tentatively identified as *Hormodendrum resinae* was isolated from a variety of creosoted wood products collected over a large territory in the United States. *H. resinae* appears to be a universal inhabitant of creosote and coal tar treated wood in service.

It is unlikely that this fungus is a common saprophyte in soil, on untreated wood, or plant refuse, since it is unable to survive well in competition with common molds. Resinous bark and wood possibly formed the original source of inoculum although other sources, as yet undiscovered, may exist.

H. resinae not only tolerates a far higher concentration of creosote and coal tar than other fungi but is able to grow and reproduce with no other source of nourishment than occurs in these compounds. It appears to be capable of metabolizing some of the components of creosote and coal tar.

H. resinae is no more resistant to inorganic toxicants than a number of other fungi.

It appears to have no effect on the strength of the wood it infects.

An unidentified bacterium was isolated from a large majority of the samples of creosoted wood from which isolations were made.

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DORMANCY AND APICAL DOMINANCE IN POTATO TUBERS ¹

H. David Michener

IT IS well known that potato tubers have a rest period lasting for two or three months after harvest, during which they will not sprout even if placed in a favorable environment. Denny and Miller (1938), Stuart and Milstead (1934), and others have shown that treatments with ethylene chlorohydrin break this rest period and produce growth in ten to twenty days after treatment. It was also shown by Michener (1941) that ethylene chlorohydrin treatments ac-

celerate germination not only in completely dormant tubers but in ones which are starting to germinate after having passed through their rest period and in ones which are starting to germinate as a result of a previous ethylene chlorohydrin treatment.

Werner (1931) has shown that ethylene chlorohydrin treatments also increase the number of buds or eyes which grow into stems. This has been confirmed by Michener (1941), who showed that the increased number of stems caused by treatment may result in an increase in yield, at least under certain conditions. As shown in table 1, the increase in the number of stems per tuber is greatest if the tubers are starting to grow when treated, either as a result

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TABLE 1. *Effect of ethylene chlorohydrin on the number of stems produced by potato tubers.*^a

Condition of tubers	Average number of stems per tuber emerging from soil		
	Untreated control	3-day treatment (0.9 cc. chlorohydrin per kg. of potatoes)	3-day treatment, 11 days storage, ^b 1½-day treatment
Dormant (17 days after harvest).....	1.4	1.7	3.2
Dormant (46 days after harvest).....	1.4	2.0	4.7
Non-dormant (buds less than 1 mm. long).....	1.6	3.6	..

^a Summarized from data presented by Michener (1941).^b When the second treatment was made the buds were about 1 mm. long.

of a previous treatment with chlorohydrin or because they have completed their rest period (see fig. 1).

In the untreated tubers, growth occurs only in buds which are near the apex of the tuber (Apple-

man, 1918). This may be due to dominance of the apical buds over lateral buds, or it may be because the apical buds have a shorter rest period and, therefore grow first. In the former case apical and lateral

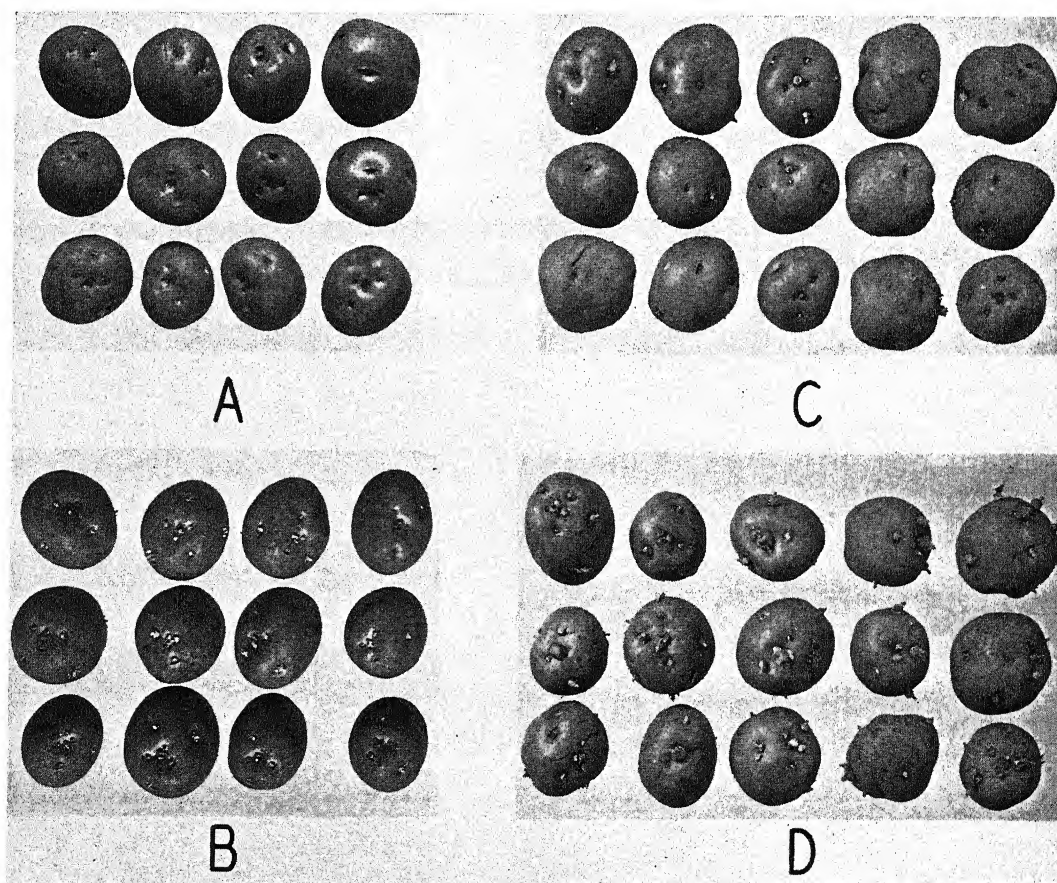


Fig. 1. A. Untreated dormant Bliss Triumph tubers, thirty-one days after harvest. B. Similar tubers to A, treated for two days with 0.9 cc. ethylene chlorohydrin per kg. of tubers. Treatment made fourteen days before photograph was taken. Most of the buds show growth. C. Untreated Bliss Triumph tubers, about fifty days after harvest. Most of the eyes which have grown are near the apices of the tubers. D. Similar tubers to C, treated for 1½ days with 1.0 cc. ethylene chlorohydrin per kg. of tubers. Treatment made twenty-one days before photograph. Buds are larger than in C, and more have grown than in C. The position of the apex of the tuber may be determined by the more or less compact group of buds around it. For example, in group C the tuber in the lower right hand corner has its apical end towards the camera while the one in the lower left hand corner is turned on its side with the apex hidden. Most of these tubers have the apex turned towards the camera.

buds should grow synchronously if they are separated by cutting the tuber; but in the latter case the apical buds should grow sooner, even if separated from the lateral buds.

The relative vigor of apical and basal seed pieces has been studied by several workers, including Stuart, Edmundson, Lombard, and Dewey (1927) and Westover (1930), who also review earlier work on the subject. Although earlier authors disagreed in some cases, the authors mentioned were unable to find any constant difference in yield between plants grown from apical and those grown from basal seed pieces.

Appleman (1918) cut potato tubers transversely in two and found that the basal half grew sooner than the apical half. However, the apical buds grew in the apical halves of the tubers, while in the basal halves the buds which grew were usually close to the cut surface.

Since Thornton (1939) states that removal of tissue near a bud causes that bud to grow in advance of others on the same tuber, it is possible that the outcome of Appleman's experiments was affected by the proximity of the lateral buds to the cut surfaces.

In the present paper it is shown that apical and lateral seed pieces germinate at approximately the same time if they are cut out of the tuber in such a way that apical and lateral buds each bear the same spacial relationship to the cut surfaces around them. It is concluded that the buds in the apical region of the tuber (hereafter referred to as apical buds) do inhibit growth of the lateral buds. This inhibition is shown to be brought about by auxin produced in the apical buds.

It is also shown that ethylene chlorohydrin treatments cause destruction of a large proportion of the auxin in the tuber. As the dominance of the apical buds depends on auxin which they produce, it is lost when the auxin is destroyed. The lateral buds are then able to grow. The possibility is also considered that this destruction of auxin is also responsible for the breaking of dormancy after ethylene chlorohydrin treatment.

METHODS.—The ethylene chlorohydrin treatments were carried out by the method previously recommended by Michener (1941), in which the tubers are placed in a closed chamber containing a dish of liquid ethylene chlorohydrin, which evaporates and is taken up by the tubers. For each kg. of potatoes 1.0 cc. of ethylene chlorohydrin was used, and the treatments were made at room temperature and lasted for three days, except where otherwise mentioned.

The varieties used were Bliss Triumph, White Rose, and Russet. All appeared to behave similarly in experiments concerned with dormancy and reaction to ethylene chlorohydrin treatment.

For auxin extractions (by the method of van Overbeek, 1938) cubes of fresh potato tissue having a volume of about 1 cc. were placed over night in peroxide-free ether in a refrigerator. After the ether was poured off, the tissue was rinsed with a second

portion of peroxide-free ether which was then added to the first. The ether was evaporated down to about 1 cc., added to a vial containing a measured amount of 1.5 per cent agar, and evaporated, leaving the auxin in the agar. The auxin was then determined by the standard *Avena* test.

From appropriate control tests it was possible to calculate the concentration of auxin (as indoleacetic acid) in each sample of tissue by the method suggested by van Overbeek and Bonner (1937). In the experiments in which this calculation was not made, the auxin was taken up in a volume of agar which was proportional to the weight of the sample. In these cases the curvature obtained in the *Avena* test, therefore, represents the concentration of auxin in the potato tissue, within any single experiment.

When the potato cubes were sliced (about 2 mm. thick) and dried before extraction, less auxin was obtained. When they were similarly sliced and extracted without drying the amount of auxin obtained was slightly less than when the tissue was extracted without being sliced.

For each extraction, 50 to 100 grams of tissue was used. Except where otherwise mentioned, this was a composite sample taken from five to fourteen tubers.

In order to determine how much of the auxin was extracted by this method, potato cubes were placed in ether for two hours, removed to a second, third, and fourth portion, each for two hours, and a fifth for eight hours. The first experiment was carried no farther, while in a second the potato cubes were placed in a sixth portion of ether for two hours and in a seventh for twenty-four hours. All ether portions were found to contain auxin, showing that auxin continued to diffuse out of the potato cubes. In the first experiment (with Russet tubers) the rate of diffusion out of the tissue fell to about 12 per cent of its initial value while in the second (with White Rose tubers) the rate only fell slightly during the experiment. The reason for this difference is not known. However, within each one of the experiments described below, all tubers were of the same variety, from the same source, and similar in size and appearance. Hence, it is believed that, within any one experiment, results of different auxin extractions are comparable.

EXPERIMENTAL RESULTS.—*Interrelationship between buds in dormant tubers.*—In order to determine whether the apical buds actually inhibit growth in the lateral buds, or whether they merely have a shorter rest period, a number of tubers were prepared as follows: Two cubes of tissue, about 1 cm. on a side, were removed from each tuber in such a way that one contained an apical bud and one contained a lateral bud, each with the skin surrounding it. At the start of the experiment the buds were about 0.1 mm. in length. These were placed for a day in strongly aerated water, as previous experiments had shown that potato pieces of this size grew better after such treatment. After this they were placed on wet filter paper in a moist chamber. When this ex-

TABLE 2. *Growth of isolated apical and lateral buds.*

Length of bud	Percentage of seed pieces falling into each class					
	Dormant (Bliss Triumph)				Non-dormant (Russets)	
	After 30 days		After 43 days		After 16 days	
	Apex	Side	Apex	Side	Apex	Side
None	43	32	38	5	0	0
0-2 mm.	43	42	43	37	0	31
2-10 mm.	9	16	14	47	42	63
Over 10 mm.	5	10	5	11	58	6
Difference between apex and side. ^a	No significant difference.		Significant (P. between .05 and .02)—side pieces grew more.		Highly significant (P.<.01)—apical pieces grew more	

^a As determined by χ^2 test.

periment was done with Bliss Triumph tubers, which were completely dormant at the start of the experiment, there was no difference between the growth of the apical eyes and that of the lateral eyes after thirty days. After forty-three days, the lateral eyes had grown slightly more (table 2). The same experiment was done with Russet potatoes, in which the apical buds were showing slight growth (less than 0.5 mm.). As was expected in this case, the apical buds grew faster than the lateral buds. Similar experiments with potatoes which had first been treated with ethylene chlorohydrin gave the same result except that growth began sooner.

As has been pointed out previously, cutting the tubers causes the buds to grow sooner than if they were not cut. As all of the pieces were cut in the same way, however, this effect should not influence the outcome of the experiment.

There is thus an obvious similarity between the potato tuber, in which lateral buds grow if separated from the terminal buds, and the pea seedling, in which lateral buds grow if the terminal bud is removed. In the pea seedling, auxin produced by the terminal bud inhibits growth of the lateral buds (Thimann, 1939). It may also be true in the potato that the apical buds produce auxin which inhibits growth in lateral buds. The following experiments were designed to test this hypothesis.

In order to study the distribution of auxin in the potato, tubers were divided into three parts: apex, sides, and center. The apex consisted of about one-sixth of the potato at the apical end. The side was a broad ring of tissue cut from the side but not ex-

tending deeper than one-third of the way to the center. The portions from both apex and side contained skin, buds, and the vascular cylinder. The center was the remainder, lying wholly within the vascular cylinder.

In a few cases (the Russet variety, table 3) large pieces of tissue were not used, but instead the bud was cut out with about 0.1 gram of tissue below it. Comparable pieces without buds were cut from the side and center of the tuber.

The data from these experiments (table 3, see also table 8) show that no auxin was obtained from the center of the tuber. When only the buds and tissue in their immediate vicinity were tested (Russet variety, table 3), the apical buds had more auxin than lateral buds. However, in one case the tubers were starting to grow, while in the other they were very near the end of their rest period. When the experiment was repeated with Bliss Triumph and White Rose tubers, which were known to be dormant, so little auxin was present that it was not possible to cut out enough buds so that a measurable amount of auxin could be extracted. For this reason, larger pieces of tissue were used. As auxin is absent from the center of the tuber, it must be in the vascular cylinder or in the comparatively thin cortex² surrounding it.

Examination of the potato tuber shows that the vascular cylinder is so shaped as to connect all the buds with one another (fig. 2). Therefore, if the auxin is mainly in the vascular bundles, auxin pro-

² According to Hayward (1938), the region between periderm and vascular cylinder is the cortex.

TABLE 3. *Distribution of auxin in the potato tuber.*

Variety	Auxin extracted (degrees curvature in <i>Avena</i> test)			
	Apex	Side	Side pieces without buds	Center
Bliss Triumph	6.7	6.6	..	—0.8
Bliss Triumph	2.8	3.0	..	—1.4
Russet	9.4	3.3	—1.7	—1.7
Russet (buds starting to grow).....	16.8	7.6	0.7

duced by one bud will readily be carried to others. Approximately the same situation will prevail if the auxin is in the cortex.

If the lateral buds are inhibited by auxin produced by the apical buds, it should be possible to cause similar inhibition by adding auxin artificially to the apical end of the tuber. To do this, tubers were selected

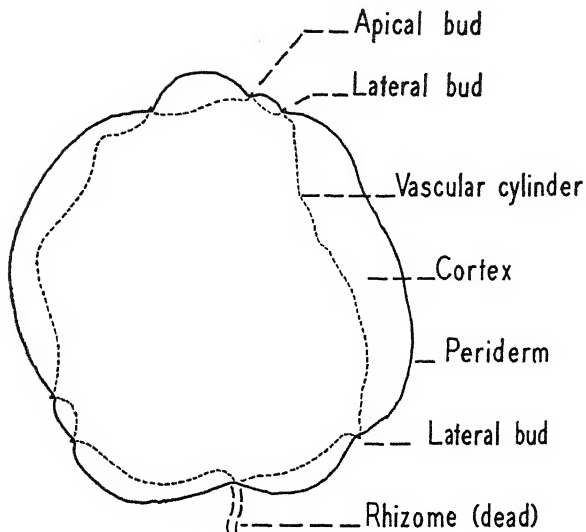


Fig. 2. Longitudinal section of Bliss Triumph potato tuber, showing relationship of buds and vascular cylinder.

which were nearly ready to come out of their rest period. One of the apical buds of each tuber (and a small area around it) was smeared with lanoline containing indoleacetic acid. Groups of ten tubers each were treated with lanoline containing indoleacetic acid in the following concentrations: 26, 3, 0.3, and 0.03 mg. per gram of lanoline. Controls were treated with pure lanoline. After six days the lanoline paste was scraped off.

When the tubers were given only the treatment described above, the auxin had no effect, although the

tubers were stored in boxes in the laboratory for several weeks. This was perhaps to be expected, however, for the apical buds inhibit the lateral buds even without the addition of indoleacetic acid.

In a parallel experiment which was done at the same time, the tubers were treated as above, but were also treated with ethylene chlorohydrin for three days immediately after the lanoline paste was applied. As shown below, this treatment removes the inhibiting effect of the apical buds on the lateral buds. If the indoleacetic acid which was added has any inhibiting effect, the lateral buds should show less growth on the tubers which were treated with indoleacetic acid than in those which were not.

After eleven days the buds were still too small for accurate measurement, but were classified as to size as follows: 0, no growth; 1, growth barely perceptible; 2, size of pin head; 3, rudimentary leaves showing; 4, bud swelling in diameter. In each tuber the following four buds were thus classified: the largest bud in the basal third of the tuber, the largest in the central third, the largest in the apical third, and the largest within 1 cm. of the apex. The addition of indoleacetic acid had no effect on the apical third of the tuber. As shown in table 4, however, it caused a significant reduction in bud growth in the middle third and basal third of the tuber.

It was mentioned above that the lanoline paste was scraped off the tubers after six days. It was subsequently applied unilaterally to *Avena* coleoptiles to estimate its auxin content. In the case of the paste containing 0.3 mg. indoleacetic acid per gram, the curvatures obtained were 14.5° for paste not applied to tubers, 13.1° for paste applied to tubers which were not treated with ethylene chlorohydrin, and 11.8° for paste applied to tubers which were also treated with chlorohydrin. For the paste containing 0.03 mg. per gram, the curvatures were, respectively, 5.8° , 4.4° , and 5.0° . Thus only a small part of the indoleacetic acid went into the tuber. As the supply did not become depleted, it may be assumed that indoleacetic acid was entering the tuber continuously

TABLE 4. Inhibition of lateral bud growth by addition of indoleacetic acid.

		Number of tubers in each classification				
	Size classifica- tion of bud	Indoleacetic acid concentration in lanoline				Control (pure lanoline)
		26 mg./g.	3 mg./g.	0.3 mg./g.	0.03 mg./g.	
Basal third of tuber.....	0	3	3	1	2	0
	1	4	4	4	2	0
	2	2	1	5	5	8
	3	1	2	0	1	2
Difference between treatment and control ^a		Significant P.<.02	Significant P.<.01	Not sig- nificant	Not sig- nificant	
Middle third of tuber.....	1	1	2	1	0	0
	2	7	5	4	6	0
	3	2	3	5	4	10
Difference between treatment and control ^a		Significant P.<.01	Significant P.<.01	Significant P.<.05	Significant P.<.01	

^a As determined by χ^2 test.

TABLE 5. *Auxin concentration in buds and tubers of treated and untreated potatoes.*

	Auxin concentration, γ per kg. of tissue	
	Untreated tubers	Treated tubers
A. Four weeks after treatment:		
Buds	87	66
Apex of tuber (without buds)	6.6	6.2
Side of tuber (without buds)	3.4	6.7
B. Immediately after treatment: ^a		
Apex of tuber (with buds)	1.6	.95
Side of tuber (with buds)	1.8	.95

^a The buds were too small to analyze separately.

until the paste was removed (and for some time after, as not all the paste could be removed).

Interrelationship of buds in non-dormant tubers.—It would be desirable to know the auxin content of the buds during early stages of growth, but it has not been possible to determine this because of the small size of the buds. Such determinations were made, however, on Russet potatoes which were stored in the laboratory until the largest buds reached a length of about 3 mm. From five tubers all buds which were growing were removed. They had a total weight of 0.53 g. and were classified as six large, nine medium sized, and twenty-four small. The large ones were all from the apices of the tubers. Besides the buds, pieces of tissue taken from the apex and sides of the tubers were analyzed for auxin. The buds contained a large amount of auxin (table 5), the apex of the tuber (beneath the large buds) contained less auxin, and the sides the least auxin.

The experiment was also done with similar tubers which had been treated four weeks earlier with ethylene chlorohydrin. Here five tubers had fifteen large buds, twelve medium sized ones, and three small ones, totaling 2.07 grams in weight. The large buds were not confined to the apex of the tubers, but were formed on the sides as well. In this case the auxin content in the side portions of the tubers was the same as in the apices.

It has thus been shown that in the untreated tuber the buds contain more auxin than the tissue beneath the buds, and that the tissue beneath the large buds contains more than that beneath the small buds.

In order to show directly that large buds inhibit smaller buds, twenty-four dormant Russet tubers

were treated with ethylene chlorohydrin to make a number of buds start to grow on each tuber. A week later, the six largest buds on each tuber were numbered from 1 to 6 in order of decreasing size. The tubers were then placed for three weeks in moist sand in a dark room, after which they were washed free from sand and the stems were measured. The measurements (excluding a few injured or abnormal stems) are summarized in table 6. Most of the buds either did not grow (remaining shorter than 10 mm.) or grew rapidly (exceeding 100 mm. in length). All of the no. 1 buds grew, but, from no. 2 on, the percentage of buds which failed to grow became larger and larger.

After the stems were measured, all stems over 10 mm. long were removed from several of the tubers. These tubers were then replanted. Within a few days other buds, which had previously remained small, began to grow.

Thus, in each tuber the growth of the smaller buds was inhibited by larger buds. This occurred in a treated tuber where several buds had started to grow. In the untreated tuber, where only one or two apical buds start to grow, it is to be expected that growth of the remaining buds will be inhibited.

Effect of ethylene chlorohydrin on auxin in tubers.—The foregoing experiments indicate that auxin plays a rôle in the inhibition of lateral buds by apical buds. As this apical dominance disappears when dormant tubers are treated with ethylene chlorohydrin, it is possible that the action of ethylene chlorohydrin on the tuber is related in some way to auxin. For this reason, the effect of ethylene chlorohydrin on the

TABLE 6. *Bud inhibition in potato tubers.*

	Mean initial length, mm.	Percentage in each size group after three weeks			Mean length after 3 weeks, mm.
		Less than 10 mm.	10 to 100 mm.	More than 100 mm.	
Largest bud	5.5	0	0	100	247
Second largest bud	4.3	9	13	78	223
Third largest bud	3.4	29	5	66	124
Fourth largest bud	3.4	50	4	46	118
Fifth largest bud	2.7	64	27	9	58
Sixth largest bud	2.3	62	33	5	31

TABLE 7. *Effect of ethylene chlorohydrin on the auxin content of the potato tuber.*

Variety	Duration of treatment	Part of tuber	Auxin extracted (degrees curvature in <i>Avena</i> test)			Duration of storage period following treatment
			Untreated controls	At end of treatment	At end of storage period following treatment	
Russet	1 day	Apex and side	7.3	0.0	8.7	2 days
Bliss Triumph	14 hours	{ Apex	..	-0.2	..	3 days
		{ Side	..	-0.2	..	
	22 hours	{ Apex	4.4	-1.3	..	
		{ Side	3.4	-0.4	..	
	3 days	{ Apex	9.3	3 days
		{ Side	7.2	
Bliss Triumph	3 days	{ Apex	6.7	3.4	11.1	2 days
		{ Side	6.6	-0.3	9.4	
		{ Center	-0.8	-1.0	-1.1	
Russet	3 days	{ Apex	10.2	2.7	..	3 days
		{ Side	13.7	2.7	..	
Russet	3 days	{ Apex	1.6	..	9.0	3 days
		{ Side	1.8	..	11.7	
White Rose	3 days	Apex and side	10.5	0.1	..	3 days
White Rose	5 days	Apex and side	5.5	2.3	5.4	

auxin content of potato tubers was made the subject of further experiments.

Tubers were treated with 1 gram ethylene chlorohydrin per kg. of tubers for periods of one to five days. Immediately after treatment they were analyzed for auxin. At the same time an analysis was made of similar but untreated tubers, and usually of a third group of tubers, which had been treated two or three days earlier in order to allow a storage period between treatment and analysis. The results of these analyses are shown in table 7 (see also table 5, part B).

This experiment shows that the auxin content was greatly decreased by the treatment, and that it rose again within two or three days after the end of the treatment. In all but two experiments the auxin content two or three days after treatment was considerably higher than in the untreated controls. Of these two exceptions, one was treated for one day and one for five days. These two treatments were shorter and

longer, respectively, than the optimum treatment of two or three days; for it has been shown (Michener, 1941) that one-day treatments do not accelerate germination as much as two- or three-day treatments, while treatments lasting four days may have an injurious effect, killing some of the tubers.

It is possible that ethylene chlorohydrin increases the rate at which auxin is destroyed in the tuber. Its effect on auxin destruction was determined by placing blocks of potato tissue, basal side down, on agar blocks containing a known amount of auxin. These blocks of potato tissue had previously been placed for two hours on wet filter paper to remove any auxin which might diffuse out. As shown in table 8, more auxin was destroyed by the treated tissue than by the untreated.

As the auxin reappears after the end of treatment, it is of interest to know where it comes from. As shown under "Discussion," there are several reasons for thinking it may be produced by the buds. Accord-

TABLE 8. *Effect of ethylene chlorohydrin on destruction of auxin by potato tissue.*

Variety	Time of contact with agar block	Auxin in agar block (Degrees curvature in <i>Avena</i> test)		
		Final concentration		
		Original concentration	Ethylene chlorohydrin treated	Untreated control
Bliss Triumph	55 min.	12.7	3.9	6.9
Bliss Triumph	60 min.	...	0.8	2.2
Russet	50 min.	3.6	0.8	3.0
White Rose	50 min.	3.6	1.4	2.3

TABLE 9. *Effect of removal of eyes on auxin formation in the potato tuber.*

Pre-treatment of tuber	Auxin content, γ per kg. of tissue		
	No treatment	Analyzed immediately after ethylene chlorohydrin treatment	Analyzed 2 days after ethylene chlorohydrin treatment
First experiment:			
Intact tuber ^a	...	2.46
Eyes removed	1.5996
Wounded between eyes	2.58	...	2.46
Second experiment:			
Intact tuber	1.85	.84	1.98
Eyes removed	2.27	.84	1.19
Wounded between eyes	3.19	1.36	1.71

^a Analysis failed through accident.

ingly this possibility was investigated experimentally.

Tubers were treated with ethylene chlorohydrin after cutting out all the eyes, each with a small amount of tissue (about 4 cubic mm.) below it. A second group consisted of intact controls. To determine the effect of wounding, if any, a third group was prepared by cutting out pieces of tissue as in the first group, but cutting them out between the eyes, leaving the eyes intact. The tubers were then stored in a moist atmosphere for a few days to permit suberization of the cut surfaces. They were then treated as shown in table 9. In the first experiment the ethylene chlorohydrin treatment lasted two days, and in the second, one day. In the first experiment each auxin analysis was made on a composite sample of three tubers, and in the second only one tuber was used for each test.

In each of these experiments, the tubers with eyes removed had less auxin two days after treatment than did those which were left intact or those which were wounded between the eyes. The regeneration of auxin after treatment in the tubers without eyes was thus either totally lacking or lower than in the tubers with eyes.

With the possible exception of the last experiment, this work does not preclude the possibility that the reduced *Avena* curvatures obtained from treated tubers were due, not to destruction of auxin, but to ethylene chlorohydrin which might have been extracted along with the auxin and brought into the agar blocks in sufficient quantity to reduce the sensitivity of the *Avena* test. As the tubers were treated with 1 cc. of chlorohydrin per kg., they could have contained a maximum of 0.1 per cent chlorohydrin if all was absorbed and retained in the tuber. Accordingly an appropriate amount of indoleacetic acid was added to 90 cc. of a 0.1 per cent (by volume) ethylene chlorohydrin solution. A control was made with the same amount of indoleacetic acid but with water instead of chlorohydrin. These solutions were then shaken with 90 cc. of ether for half an hour on a shaking machine. This was repeated with a second portion of ether, which was then added to the first.

The ether was then evaporated and tested for auxin in the same manner as were the ether extracts from potatoes. The *Avena* curvatures obtained for the chlorohydrin solution and the control were, respectively, 16.7° and 19.5°. When part of the agar was diluted with an equal volume of pure agar the curvatures were 11.4° and 11.3°, respectively. A second experiment gave similar results. This eliminates any possibility that the ether extracts of treated potatoes could contain enough chlorohydrin to affect the auxin determinations.

Experiments were also done on the effect of ethylene chlorohydrin *in vitro*. Agar blocks were made containing 0.1 per cent chlorohydrin as well as auxin. These were tested for auxin, along with controls containing only auxin. One test gave curvatures of 20.7° with chlorohydrin and 19.7° without. With half as much auxin the curvatures were, respectively, 17.3° and 11.2°. In another experiment a solution containing auxin and 0.1 per cent chlorohydrin was allowed to stand for twenty hours. When tested it gave a curvature of 14.1° and the control gave 11.4°. Certainly, therefore, 0.1 per cent ethylene chlorohydrin does not decrease the *Avena* curvature or destroy indoleacetic acid *in vitro*. This also shows that the data presented in table 8 could not result from a direct effect of chlorohydrin on the *Avena* test.

DISCUSSION.—As described above, the terminal buds of an untreated potato tuber grow sooner than the lateral buds. This has been shown to be a correlation phenomenon rather than a fundamental difference between the buds themselves; for all buds grow at the same time if they are isolated from each other while completely dormant.

In this respect the potato tuber resembles growing stems such as those of pea seedling, where it is known (Thimann, 1939) that auxin produced by the terminal bud inhibits growth of the lateral buds. In the potato tuber, as in the pea stem, the buds are connected to one another by means of the vascular tissue. It has been shown that the auxin is present in the vascular tissue or in the cortex, where it is in close proximity to the buds.

The potato tuber also resembles the pea stem in that lateral bud growth may be inhibited by indoleacetic acid added to the apex. There is a difference in the method by which this can be carried out, however. In the pea stem, the natural source of auxin can be removed by cutting off the apical bud. Then the lateral buds grow unless indoleacetic acid (or another auxin) is supplied artificially to the apex. In the potato tuber cutting off the apex will, in itself, affect bud growth (Appleman, 1918). It is possible, however, to bring about destruction of a large part of the auxin by treating the tuber with ethylene chlorohydrin. After this treatment, indoleacetic acid applied to the apex has an inhibiting effect on the growth of the lateral buds.

This analogy between potato tuber and pea stem is of significance only if the auxin in the tuber is produced in part, at least, by the buds. This appears to be the case, for intact tubers regenerate auxin completely within two days after ethylene chlorohydrin treatment, while tubers from which the eyes have been removed regenerate little or no auxin in this length of time.

Evidence from other sources also supports this view. Rosa (1928) has shown that, while potato buds appear dormant, they actually enlarge to a considerable extent during the resting period, if the tubers are stored at a temperature favorable for growth. It is, therefore, reasonable to suppose that they produce auxin, especially in view of the work of Avery, Burkholder, and Creighton (1937) and of Bennett and Skoog (1938) showing that buds of deciduous trees begin to produce auxin in the spring before enough growth has occurred to produce visible swelling of the bud.

As the apical buds in the dormant tuber appear similar in size to the lateral buds, it is not clear how they can produce sufficient auxin to inhibit the lateral buds. However, Rosa (1928) states of a White Rose tuber which is "nearly full grown but still immature" that as this variety "shows strong apical dominance, the apical sprout is much further developed than any other on the tuber." If the apical bud is larger than the lateral buds, it probably produces more auxin than the lateral buds. In the potatoes used in these experiments, where the bud which grew was near but not always at the morphological apex, one or several of the apical buds might be larger than the lateral buds.

It may also be of significance that the terminal end of the tuber contains many more eyes than any other part. It may be seen from figure 1 that a large proportion of the eyes (perhaps 50 per cent) are in the apical region.

Also, the auxin in the potato tuber may be transported from apex towards base, as in many stems. If so, it is to be expected that auxin produced by apical buds will move towards lateral buds. It has not been possible to demonstrate polar transport of auxin in potato tissue. The rapid destruction of auxin by potato tissue, and perhaps also the small amount of

vascular tissue, make this phenomenon difficult to demonstrate in the potato tuber.

The relationship of apical and lateral buds has not been shown with certainty in the tuber which is just emerging from dormancy. It is much clearer after growth has proceeded to a point where the buds are several mm. long. At this stage large buds inhibit small ones, even when there is only a two or three-fold difference in size. In a normal, untreated tuber which has just germinated, there is a much greater difference in size than this between apical and lateral buds.

The auxin concentration in the growing buds at this stage is about ten times as high as in the apical tissue below the large buds, and about twenty times as high as in the tissue below the lateral buds, in which growth is inhibited. Thus the auxin concentration in the tuber decreases with distance from the apical buds. This is the condition which is necessary, according to Went (1939), to divert growth-promoting substances from the small, lateral buds to the large apical buds, thereby inhibiting growth of the lateral buds.

The experiments thus far discussed relate to inhibition of lateral buds by terminal buds in untreated tubers. The principles involved, however, appear to be related also to the effect of ethylene chlorohydrin on dormancy. It has been shown that ethylene chlorohydrin causes disappearance of much of the auxin in the tuber, and that the auxin reappears soon after treatment. This auxin which appears after treatment is produced in part, at least, by the buds.

Auxin produced after treatment will be transported from the buds into the surrounding tissue of the tuber. As this tissue has been depleted of auxin by the effect of the ethylene chlorohydrin, the new auxin coming from each bud will produce an auxin concentration gradient around the bud, with a high auxin concentration in the bud and a low concentration at some distance from the bud. According to the previously mentioned theory of Went (1939), this is the condition which would bring about growth of the buds, for it will bring about translocation towards the buds of materials necessary for growth. This concentration gradient around the buds either did not exist or was of less magnitude before treatment, for much more auxin was then present in the tuber.

It may then be asked why the tuber does not again accumulate auxin until bud growth is inhibited. Probably the initial stages of growth are accompanied by increased auxin production, and as the growth-rate increases the auxin production also increases, thus always maintaining the auxin concentration in the bud at a higher level than in the tuber. This view is supported by the fact that within two or three days after treatment the auxin concentration is often higher in treated tubers than in untreated controls.

It is of interest to note that Guthrie (1939) found that treatment of tubers or cut seed pieces with any of several different auxins prolonged dormancy, and even produced dormancy in tubers which would otherwise have grown immediately when placed in a

favorable environment. This dormancy could be broken by treatment with ethylene chlorohydrin. In view of the experiments described here, it may be concluded that ethylene chlorohydrin treatment causes destruction not only of naturally occurring auxin but of auxins artificially added to the tuber or brought into contact with cut surfaces of the tuber.

This hypothesis may also account for the fact that ethylene chlorohydrin accelerates bud growth even in tubers where the largest bud has reached a length of 1 mm. (Michener, 1941). Destruction of the auxin within the tuber will increase the steepness of the auxin concentration gradient between the bud, which produces auxin, and the tissue of the tuber, which has an abnormally low auxin content after treatment. According to Went's theory, this should increase the rate of translocation of growth-promoting substances towards the bud.

These experiments also agree with the previously stated hypothesis that apical dominance in germinating, untreated tubers is the result of auxin produced by the buds at the apex of the tuber. If this is correct, then disappearance of the auxin during treatment will cause disappearance of apical dominance and allow each bud to act independently. After treatment, of course, larger buds eventually produce enough auxin to inhibit the smaller ones; but, as shown in table 6, there are usually several buds which continue to grow in a treated tuber.

Guthrie (1939) also extracted auxin from potatoes after ethylene chlorohydrin treatment. As his extractions were made three to seven days after treatment, he failed to detect the sudden disappearance of auxin during treatment. In experiments in which he used the extraction technique of van Overbeek (1938), the auxin concentration in the treated tubers approximately equalled that in the controls by the time he made the extraction, which agrees with the experiments described here. The extraction technique of du Buy (1938) gave much lower auxin concentrations, and in this case the auxin content of the treated tubers was higher than that of the controls.

No experiments have been done by the author to determine the conditions which bring about the end of the rest period in untreated tubers. As Rosa (1928) has found that the buds continue to grow even during the rest period, it may be suggested that they finally produce auxin rapidly enough to cause movement of growth-promoting substances towards the bud.

It has been shown by Bushnell (1929) and Werner (1931) that apical dominance gradually disappears

if potatoes are in cold storage for several months. Experiments have not been done on this point either, but it is possible that buds fail to grow and produce auxin at a low temperature. If auxin destruction continues, this could lead eventually to depletion of the auxin in the tuber and consequent loss of apical dominance.

It is of interest to compare these experiments on ethylene chlorohydrin with those on ethylene (Michener, 1938). It was shown that ethylene causes auxin destruction at cut surfaces in pea seedlings, as does ethylene chlorohydrin in potato tubers.

SUMMARY

When the intact potato tuber begins to grow after its rest period, one or more apical buds grow, but the lateral buds usually fail to grow. If, however, lateral buds and apical buds are cut out and grown separately, both start to grow at the same time.

In non-dormant tubers, any large bud usually inhibits growth of smaller ones.

In non-dormant tubers, the auxin concentration is high in the large apical buds, lower in the tissue below them, and lowest in the tissue below the lateral buds.

In tubers in which apical dominance has been destroyed by treatment with ethylene chlorohydrin, application of indoleacetic acid to the apex of the tuber causes inhibition of the lateral buds. In other words, apical dominance can be produced artificially by applying indoleacetic acid to the apex of the tuber.

Therefore, it is probably auxin produced by the apical buds which correlates growth of apical and lateral buds when the tuber begins to grow after its rest period.

When the tuber is treated with ethylene chlorohydrin, much of its auxin disappears. Special experiments show an increase in auxin destruction at the time. The auxin reappears within two or three days after the end of treatment.

It is suggested that auxin inhibits bud growth in the dormant tuber, and that removal of the auxin by the action of ethylene chlorohydrin permits growth to proceed.

The disappearance of apical dominance which follows ethylene chlorohydrin treatment is also a result of the auxin destruction which takes place during the treatment.

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INTERACTIONS OF AUXINS IN GROWTH AND INHIBITION¹

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ALL KNOWN substances with auxin activity possess several common structural properties (*cf.* Koepfli, Thimann and Went, 1938). A few highly active compounds have been reported to have approximately equal activities in the pea test, and perhaps also in the *Avena* test when corrections are made for differences in transport rates (Thimann, 1935) and dissociation constants (D. Bonner, 1938), but many compounds remain which have a definitely lower order of activity even after such corrections are made. Thus in general the quantities of different substances required to produce a given response vary over a wide range.

Substances with structures indicative of auxin activity may also exhibit apparently qualitative differences in a given test method. Thus γ -phenylbutyric acid is almost inactive in the standard *Avena* test; it produces, at most, a slight increase in growth in section tests, and only small curvatures in the pea test. However, when plants are first treated with phenylbutyric acid and then some hours later with indoleacetic acid in the pea test (slit stem test), the response to low concentrations of indole-3-acetic acid will be very markedly increased (Went, 1939a) (see also Went 1939b and c). On the basis of this increase in activity, Went has proposed that phenylbutyric acid is a "hemi-auxin," *i.e.*, it possesses only those properties of an auxin which are effective in the first steps leading to growth. In this explanation two assumptions are made: (1) the activity of auxin is exerted in at least two separate reactions, and (2) different structural properties of the compounds are involved in the two reactions.

It follows from Went's interpretation of his pretreatment data either that indoleacetic acid (and also the auxin present in plant tissues) has the properties

required for the after-treatment to a higher degree than it has those required for the pretreatment, or that only a small proportion of the number of molecules taking part in the pretreatment reaction are needed for the after-treatment reaction. It is a necessary consequence that the application of indoleacetic acid in combination with phenylbutyric acid as well as in a separate pretreatment should be more effective than the application of the same concentration of indoleacetic acid alone. No data are available on this point. On the other hand, van Overbeek (1938), using the *Avena* test, has presented results, the quantitative significance of which is based on the assumption that the presence of phenylbutyric acid does not affect the magnitude of curvatures produced by indoleacetic acid or by auxin extracted from pea plants.

A further study of the effects of phenylbutyric acid on the growth promoting activity of indoleacetic acid is of interest both from theoretical and practical standpoints. Results obtained with various test methods are therefore presented below: These confirm Went's pretreatment results but show in addition that when phenylbutyric acid is supplied simultaneously with indoleacetic acid it markedly inhibits the activity of the latter. An explanation of this inhibition as well as of the stimulating effects of pretreatments with phenylbutyric acid is suggested in terms of quantitative rather than qualitative differences in activity of the two substances.

MATERIALS AND METHODS.—Combinations of serial dilutions of indole-3-acetic acid (IA) and γ -phenylbutyric acid (ϕ B) were tested by the following methods: (1) ordinary *Avena* tests (*cf.* Went and Thimann, 1937); (2) straight growth of decapitated coleoptiles supplied with the substances from agar blocks covering the cut surfaces (*cf.* Went,

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TABLE 1. *Avena* test curvatures, in degrees, for combinations of γ -phenylbutyric (ϕB) and indole-3-acetic (*IA*) acids.

Experiment I				Experiments II and III			
ϕB , mg./l.	0	<i>IA</i> , mg./l.		ϕB , mg./l.	0	<i>IA</i> , mg./l.	
		0.008	0.04			0.03	0.5
0	—	2.1	10.5	0	—	21.2	24.4
2	—	^a +0.2	2.2		—	23.2	23.3
5	—	0.0	1.7	10	+0.4	10.9	24.3
50	0.0	1.3	1.9	50	+0.1	12.2	20.1
					0.1	6.7	21.5
					0.6	6.1	19.9

^a+ Indicates curvatures toward the side of the block.

1935); (3) straight growth of coleoptile sections immersed in solutions with and without added sugar (cf. Schneider, 1938); (4) auxin transport tests with sections of *Tradescantia* internodes (cf. van der Weij, 1932); and (5) ether extraction of auxin (cf. Thimann and Skoog, 1940).

All experiments in which measurements of growth were made, including curvatures, were carried out with seedlings of *Avena sativa*, variety Victory, in a darkroom at 25°C. and 85 per cent relative humidity. Red light was used for illumination. Concentrations of auxin are quoted as milligrams per liter of medium (i.e., of distilled water or of 1.5 per cent agar). The volumes of the agar blocks were approximately 10 cubic millimeters each.

RESULTS.—*Avena* test curvatures.—Curvatures obtained with indoleacetic acid and phenylbutyric acid in the standard *Avena* test are shown in table 1. The most striking effect of phenylbutyric acid is that, instead of increasing the response to indoleacetic acid, it decreases or may completely prevent the curvatures normally obtainable from a given concentration of indoleacetic acid.

It may be seen further that phenylbutyric acid alone in the concentrations used in this experiment produces no significant curvatures. However, traces of activity (average ca. 1 degree) are frequently obtained with concentrations of 10 mg./l. or more. These curvatures are confined to the upper 2 or 3 mm. of the coleoptiles. Went (1939b) attributes such curvatures to the "hemiauxin" activity of phenylbutyric acid. If so, however, we should expect to have longer curved zones more nearly equal to those

produced by the auxin from the plant itself, because the transport of phenylbutyric acid is not restricted to short distances from the tip. The very short curved zones suggest rather that the phenylbutyric acid itself may have some auxin activity in very high concentrations (cf. Thimann and Schneider, 1939), or else that it exerts an acid effect (cf. J. Bonner, 1934).

The inhibiting effect of phenylbutyric acid on indoleacetic acid activity has been obtained consistently in some fifteen experiments. It cannot be ascribed to toxicity, because with low concentrations of indoleacetic acid clear cut inhibition is obtained with 2 and 10 mg./l. phenylbutyric acid, whereas with higher concentrations of indoleacetic acid even 50 mg./l. phenylbutyric acid exerts little if any inhibiting effect.

On the other hand phenylbutyric acid, in a range of concentrations from 0.1 to 50 mg./l., added to various concentrations of indoleacetic acid, has in no case led to an increase in curvature over that produced by the corresponding concentration of indoleacetic acid alone. Hence, in simultaneous treatments with the two substances in the *Avena* test phenylbutyric acid can neither be considered to carry out nor to facilitate exclusively any one reaction of an auxin. On the contrary the results show that the phenylbutyric acid blocks the action of the indoleacetic acid in accordance with the relative proportions in which the two substances are supplied.

Straight growth of decapitated coleoptiles.—Since curvatures are a measure of the relative increases in length of the two sides rather than of total elongation

TABLE 2. Percentage elongation of *Avena* coleoptiles to which combinations of *IA* and ϕB were supplied in agar. The measured zone, ca. 11. mm. long, extends downward from the cut surface.

1.8 hours					14 hours			
ϕB , mg./l.	0	<i>IA</i> , mg./l.				<i>IA</i> , mg./l.		
		0.03	0.06	0.50		0.03	0.06	0.50
0	3	9	11	12	25	24	30	43
0.5	4	10	13	12	23	26	30	39
5	2	8	9	10	10	25	29	34
50	4	4	5	11	12	11	22	37

of the coleoptile, the experiments above have been supplemented by others in which the agar blocks containing phenylbutyric acid, indoleacetic acid, or mixtures of the two were applied to the entire cut surfaces of the decapitated plants. Increments in length of the coleoptiles were measured with a horizontal microscope. The results of one such experiment are

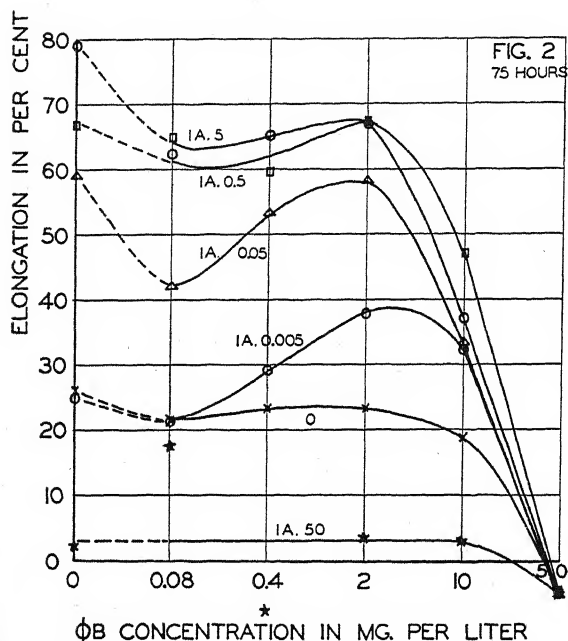
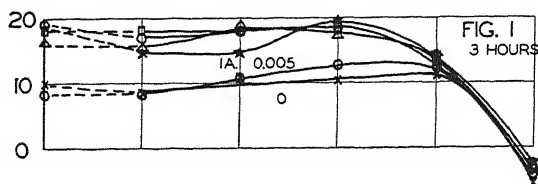


Fig. 1-2. Per cent elongation of 3 mm. long sections of *Avena* coleoptiles in the section test with 2 per cent sucrose solution and various concentrations of indoleacetic and phenylbutyric acids. (The numbers inserted represent concentrations of indoleacetic acid in mg./l.)

presented in table 2. They show that the inhibitory action of phenylbutyric acid is also obtained in straight growth measurements. The only concentration of phenylbutyric acid indicating a possible stimulation was 0.5 mg./l. and even here the effect is so small as to be of doubtful significance. The measurements after fourteen hours are not quantitative, as the applied agar blocks give up most of their activity within the first five hours. They are included, nevertheless, to show that extensive growth is possible even after application of 50 mg./l. concentrations of phenylbutyric acid provided that sufficient indoleacetic acid is also supplied. They show also a long time inhibition of growth caused by the application of phenylbutyric acid alone or in conjunction with very low indoleacetic acid concentrations. This is not mainly a general effect of toxicity

but rather a specific inhibition of auxin action and probably also of auxin regeneration in the decapitated plants.

Growth of segments of coleoptiles in solution.—Segments of coleoptiles were immersed in solutions and their growth was measured. Three successive 3 mm. sections beginning at about 5 mm. below the tips of three-day-old coleoptiles were used. The experiments were done both with and without sucrose added to the solutions.

This method of immersing the tissues in solutions approaches the conditions of the "pea test"; correspondingly, it yields results more closely in agreement with those of the pea test in that a stimulating effect of phenylbutyric acid can be obtained. As shown in figures 1 and 2, increasing concentrations of phenylbutyric acid tend to increase the effectiveness of the low and intermediate concentrations of indoleacetic acid. Only in one case, however, that of 0.005 mg./l. indoleacetic acid, does the growth with added phenylbutyric acid actually surpass that of the controls with indoleacetic acid alone. This effect, while relatively small as observed in straight growth tests, would be pronounced in a curvature test which involves small differences in elongation of closely adjacent tissue layers. It may, therefore, compare

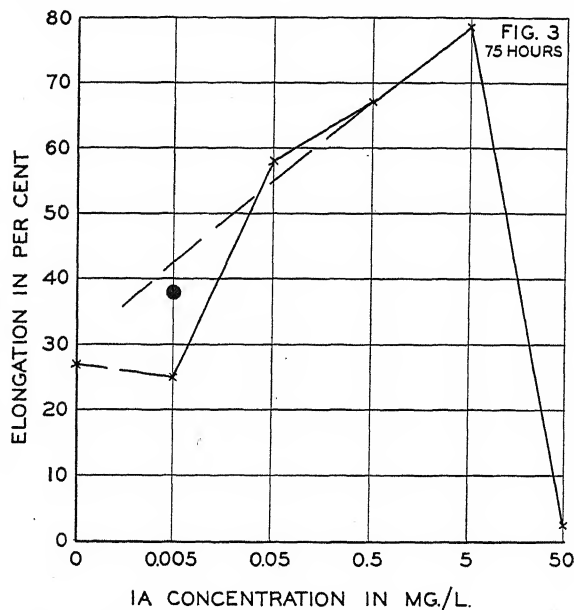


Fig. 3. Per cent elongation with indoleacetic acid (data from fig. 2. Explanation in text).

favorably with the pretreatment effect in the pea test. The concentrations used here are also very comparable with those found most effective for pretreatment, i.e., the concentration of phenylbutyric acid just below the toxic level, and the concentration of indoleacetic acid near to the threshold for increased growth. On the other hand it should be noted that these concentrations are the least suitable for determining independent qualitative effects of the two substances. The existence of a threshold value, which

is rather high for the pea test, indicates in itself that a considerable proportion of the applied indoleacetic acid is inactivated by the cut tissues, and this is corroborated by other evidence (cf. Thimann, 1934). It is possible, therefore, that the added phenylbutyric acid itself does not, except to a small extent, carry out any of the reactions leading to growth, but rather prevents destruction of the indoleacetic acid so that a greater fraction of the latter will be available for growth. This explanation is strongly supported by the results of the present experiments. In figure 3 is plotted the per cent increase in length of the sections against concentration of indoleacetic acid in treatments with this substance alone. It will be seen that the value for 0.005 mg./l. is abnormally low. In fact it appears as if this concentration had been entirely inactivated without contributing to growth. Furthermore even the highest response to this concentration of indoleacetic acid obtained by addition of phenylbutyric acid (value shown by large circle in the figure) is less than the value to be expected from the indoleacetic acid alone on the basis of the relationship between growth and concentration exhibited by the rest of the curve. It is concluded, therefore, that the increase caused by the addition of phenylbutyric acid is in large part due to its sparing action on the indoleacetic acid. This effect may become pronounced also for higher concentrations of indoleacetic acid in experiments of long duration.

A second most striking feature of the curves in figure 2 is the reduction in activity of indoleacetic acid by the low concentrations of phenylbutyric acid and the partial compensation for this reduction by the higher concentrations of phenylbutyric acid. This effect cannot be ascribed to a sparing action as above, since the same concentrations of indoleacetic acid alone produce even larger increases in growth than any of the mixtures. However, for this same reason it is also impossible to interpret the results in terms of a "hemiauxin" activity of phenylbutyric acid. This would require that the growth response to mixtures of intermediate (suboptimal) concentrations of indoleacetic acid and optimal concentrations of phenylbutyric acid should be as large as the response to the optimal concentration of indoleacetic acid alone. This result was never obtained. Here, as in the earlier experiments with the substances applied in agar blocks, there is rather a competition between the phenylbutyric and indoleacetic acids in the reaction system leading to growth. Granting this, the results of the present experiments can be interpreted in accordance with a simple general scheme for auxin action which will be presented below.

The rise of the curves in figure 2 with the higher concentrations of phenylbutyric acid cannot be explained in terms of simple competition between phenylbutyric and indoleacetic acids. These portions of the curves may, however, be included under this general scheme. For this purpose data are required regarding rates of formation and inactivation of the separate components of the auxin reaction system. The relative concentrations of these components will

be affected by the presence of phenylbutyric acid. Especially in long time experiments this may lead to changes in growth rates.

Effects of phenylbutyric acid on analyses for auxin activity.—The above experiments have made it clear that phenylbutyric acid mixed with indoleacetic acid in solutions supplied to test objects seriously reduces the activity of the indoleacetic acid in straight growth and curvature tests with *Avena*. Attempts have been made in experiments with both solutions and plant tissues containing phenylbutyric acid and indoleacetic acid to separate the active material by ether extractions, by diffusion, and by transport of the substances through plant tissues. The results indicate that the presence of phenylbutyric acid in plant tissues will prevent quantitative determinations of auxin by either extraction or diffusion methods.

1. Solutions extracted with ether.—In table 3 are shown results from an experiment in which comparisons were made of the activities of known concentrations of indoleacetic acid separately and in mixtures with phenylbutyric acid. Agar blocks for testing were prepared both directly from aqueous solutions and from ether extracts of these solutions. One cc. samples were extracted with three successive 0.5 cc. volumes of ether. The extracts were combined, evaporated to dryness and the residue was taken up in a one cc. volume of water from which agar blocks (1.5 per cent) were prepared. Some of these blocks were tested directly, others were first diluted to half the concentration by being placed in contact with equal sized agar blocks for one or two hours.

It may be seen that considerable indoleacetic acid activity was lost in the process of ether extraction. Thus the 0.03 mg./l. indoleacetic acid concentration gives 21.2° when the blocks are made directly and only 12.4° when they are made after ether extraction. (This loss is much less when larger volumes are used.)

It may be seen further that blocks made from ether extracts of mixtures of phenylbutyric acid and indoleacetic acid solutions give much lower curvatures than the extracts from the pure indoleacetic acid solution. On the whole the inhibiting effect of phenylbutyric acid was found to be relatively larger in the ether-extracted samples than in the original aqueous solutions. This is to be expected since the indoleacetic acid activity is less in the extracted samples (compare table 1). It is clear that the two substances cannot be quantitatively separated by ether extraction.

On the samples which were diluted to half concentration by diffusion into agar blocks separate tests were made on the original and added plain agar blocks. No difference in activity could be detected between the two, so that no separation of the substances was accomplished by diffusion in agar for one- or two-hour periods. (In tables 3 and 4 the mean values of both blocks are used.)

2. Plant tissues extracted with ether.—Sections of *Tradescantia* internodes were supplied with phenyl-

TABLE 3. Activities of indoleacetic and phenylbutyric acids from aqueous solutions and from ether extracts of these solutions.

Dilution	Indoleacetic acid		Agar blocks prepared with ϕ B obtained				
	conc. mg./l.	obtained from	Directly from water solns.		from ether extracts of solns.		
			conc. phenylbutyric acid in mg./l.				
			0	ca. 5	ca. 50	ca. 5	ca. 50
Avena curvatures in degrees							
..	0	+3.1	+1.2
none		water	21.2	11.0	4.8
1/2		solns.	10.8	1.3	0.5	8.4	0.5
	0.03						
none		ether	12.4	1.3	3.0
1/2		extracts	2.2	0.2	0.3	0.7	0.0
none		water	32.7	20.3	15.3
1/2		solns.	13.7	9.2	5.2	10.3	5.8
	0.06						
none		ether	25.2	19.8	7.0
1/2		extracts	12.4	3.4	1.5	3.5	4.8

butyric acid, indoleacetic acid or mixtures of the two substances either by placing agar blocks on their apical cut surfaces or by immersing the entire sections in solutions for two hours. The sections were then rinsed in running distilled water for a few minutes, dried off on filter paper and placed in ether overnight for extraction at 2°C. The ether extracts were made into agar blocks as before and tested on *Avena*. In these experiments, carried out in 1938, difficulty was encountered in making appropriate dilutions within the range of concentration covered quantitatively by the *Avena* test, because the plant material was high and variable in auxin activity. In several cases the highest dilutions used for testing were above the range of linear proportionality. Nevertheless, the results show clearly that phenylbutyric acid inhibits the activity both of the auxin extracted from the plants themselves and of the indoleacetic acid added to the tissues. This was equally true, whether the phenylbutyric acid was first supplied to the tissues together with indoleacetic acid or the phenylbutyric acid was only added subsequently to the extracts. Thus, in one experiment extracts from tissues supplied with only water or indoleacetic acid solutions gave curvatures between 31 and 24 degrees in the lowest concentrations tested, whereas the same dilutions of extracts from plants supplied with phenylbutyric acid or mixtures of phenylbutyric acid and indoleacetic acid gave cur-

vatures between 5 and 20 degrees. It is clear from these results that no separation of phenylbutyric acid from indoleacetic acid or from the auxin in the plant can be obtained by ether extraction and subsequent diffusion through agar blocks. Thus the presence of phenylbutyric acid in tissues as well as in solution will prevent a quantitative estimation of auxin activity by methods now in use. In this respect phenylbutyric acid differs from the inhibitors which have been extracted from plant tissues (Goodwin, 1939; Larsen, 1936-1939; Stewart, 1939). The method proposed by Boysen-Jensen (1941) for separating auxin quantitatively from interfering substances by diffusion, therefore, may not be of general application.

3. *Auxin transport in tissues*.—In a series of experiments the transport of indoleacetic acid and phenylbutyric acid mixtures was studied. Sections of *Tradescantia* internodes 5 mm. long were placed with their morphologically basal surfaces on wet filter paper for about two hours to remove most of their free auxin content. The sections were then placed with their bases on plain agar blocks and other blocks containing indoleacetic acid and phenylbutyric acid in different combinations were placed on their apical surfaces. After two hours the sections were removed and the basal agar blocks were cut into smaller blocks for testing. Some of these blocks were, however, first diluted to one-half concentration by diffusion into

TABLE 4. Transport of indoleacetic and phenylbutyric acids through 5 mm. long sections of *Tradescantia* internodes.

Top	IA. conc. mg./l.	0	0	0	1.1	1.1	1.1
Blocks	ϕ B. conc. mg./l.	0	ca. 5	ca. 50	0	ca. 5	ca. 50
Avena curvatures in degrees							
Dilution:							
	None	0	0	2	19(max.)	17	7
Basal	One half with agar	0	0	0	15	9	3
Blocks	One half with 0.06 mg./l. I.A. in agar	17	18	10	22(max.)	18	10
	Decrease in activity from added ϕ B	0	7	..	4	12

plain agar blocks as above, others were placed on equal sized blocks containing 0.06 mg./l. indoleacetic acid but no phenylbutyric acid. After two hours contact the blocks were separated and all were tested on *Avena* at the same time.

The results of one such experiment are presented in table 4. They show, first, that when mixtures of phenylbutyric acid and indoleacetic acid are supplied to the sections the activity obtained in the basal blocks is definitely less than when the same concentration of indoleacetic acid is supplied alone. Secondly, they show that the transport of indoleacetic acid is not prevented by the presence of phenylbutyric acid (curvatures of 17 and 7 degrees were obtained with the two concentrations of phenylbutyric acid used here); and thirdly, that the amounts of phenylbutyric acid transported through the sections are large enough, even in half concentrations, to inhibit markedly the activity of a 0.03 mg./l. concentration of indoleacetic acid in the *Avena* test. Thus the amounts of indoleacetic acid transported through the sections are also relatively large. The presence of phenylbutyric acid may have reduced the amount of indoleacetic acid transported, but the curvatures obtained indicate clearly that this effect if present must be very small. These results strikingly support the conclusion drawn from the results obtained with the "section tests" that the inhibiting effect exerted by phenylbutyric acid on the activity of indoleacetic acid is not mainly due to interference with the transport of indoleacetic acid through the plant tissues.

A mechanism for the interaction of auxins in growth and inhibition.—Results of many types of experiments, including measurements of respiration, growth, and the oxygen sensitive protoplasmic streaming, have shown that under suitable conditions indoleacetic acid will accelerate and under other conditions it will inhibit the rates of all these processes (Thimann and Sweeney, 1937; Sweeney and Thimann, 1938; Commoner and Thimann, 1941). These experiments as well as those of Schneider (1938) have shown an intimate relationship between auxin and carbohydrates and indicate that indoleacetic acid acts in fact as a respiratory coenzyme. Warburg and co-workers (Warburg, 1937) have demonstrated the chemical nature and general properties of some such coenzymes and have shown that they are functional only in combination with proteins, *i.e.*, they form reactive parts of large molecular aggregates. Evidence for the existence of auxin-protein complexes in plant tissues has also been obtained (Skoog and Thimann, 1940; Thimann and Skoog, 1940). Even though there is no proof that the fraction of auxin which is active exists in such a complex this seems likely.

Auxin activity then will depend on two separate properties: (1) structural configuration required for the molecule to occupy definite positions in the large molecular aggregate and (2) a specific chemically reactive group. That these two sets of properties are actually required and are characteristic of all known

substances with auxin activity has been clearly brought out in the studies of comparative auxin activities of benzene, indole and naphthalene derivatives (Kögl and Kostermans, 1935; Koepfli, Thimann and Went, 1938). Briefly the minimal require-

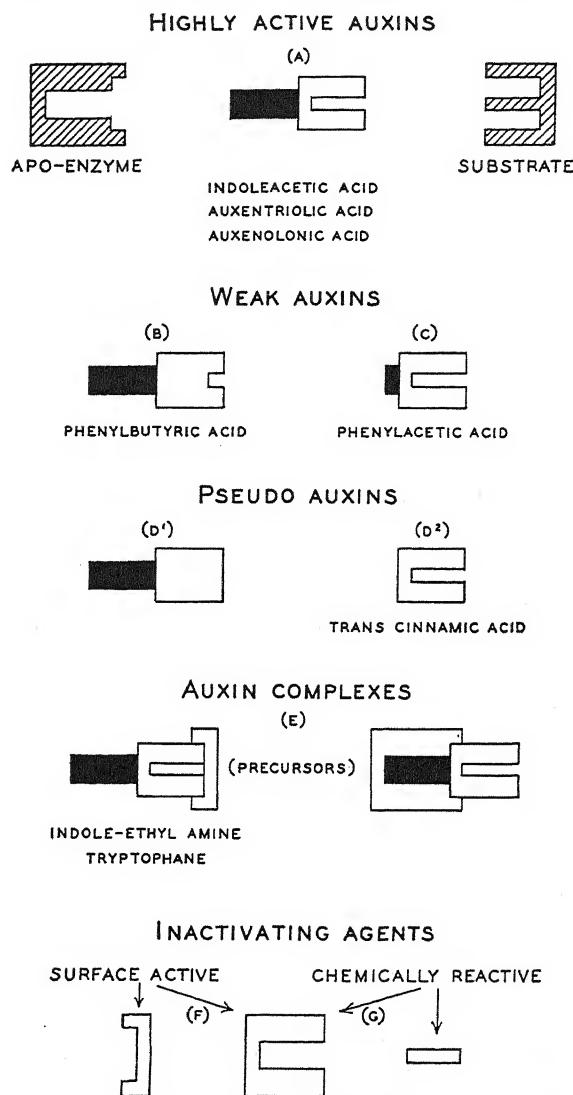


Fig. 4. Diagram representing relative activities and types of action of auxins. (Markedly inhibitory substances are on the left side, and highly chemically reactive substances on the right.)

ments include a ring system with a side chain containing at least 1 carbon atom in addition to a terminal carboxyl or potential carboxyl group, a double bond in the ring joining the carbon atom linked to the side chain, and a particular spacial relationship between the ring and the carboxyl group. These properties may be represented diagrammatically as in figure 4. A very active auxin such as indoleacetic acid or auxin A is given by (A). Here the required structural configuration is represented by a bar pro-

jecting to the left, and the chemical reactivity by the depth of the slit to the right. This distinction is of course arbitrary in that modifications in structure within the limits of the required configuration may quantitatively affect the reactivity of the molecule. Nevertheless, it serves to differentiate between two main criteria of activity. There are accordingly two types of weak auxins. One type (B) has the required structural configuration to compete successfully for positions in the reacting system but is able to react chemically only slowly. Hence, if present alone in high concentration, such a substance may cause appreciable growth, but in the presence of more active auxins it will act mainly as an inhibitor. An exception to this is where the concentration of the more active auxin is so low that the sum of the activities of the two substances, together with the sparing action of the former on the latter, will be greater than the competitive inhibitory effect. A second type (C) has high reactivity, but due to structural deficiencies it is unable to meet successfully the spacial requirements. Substances of this group, too, may be slightly active in high concentrations, but they will not act efficiently as inhibitors. Rather if they are able to react independently with available substrate groups, they may act as inactivating agents. According to this scheme "pseudo auxin" in which the double bond shifts from the ring to the side chain, thus completely removing auxin activity (Kögl, Königberger and Erxleben, 1936), will have the configuration shown in (D_1) if the double bond forms a part of the reactive group, which seems likely; or (D_2) if the double bond is merely a part of the configurational requirements. The inhibitor extracted from plant tissues and which is convertible to active auxin (Stewart, 1939; Stewart, Bergren and Redemann, 1939) would be represented by the structure (E). To this group would also belong such substances as tryptophane and indolethyl amine which likewise act as inhibitors but can be converted into active auxins (Skoog, 1937).

On the other hand, one substance, phenylacetic acid, has been reported active as an auxin in inducing parthenocarpic fruit development but completely inactive as an inhibitor of bud development (Gustafson, 1941). It can be readily seen that this behavior, which finds no explanation in earlier concepts of auxin action, would be expected as typical of substances in group (C) of the present scheme.

In addition to the above types it is of course possible to have two kinds of more or less specific inhibitors and inactivating agents as indicated in (F) and (G), which are able to combine or to react with the active substance and thus affect growth without themselves having the structure of auxins.

It is realized that a simple scheme of this nature can be only an approximate representation of the actual situation. Nevertheless, it has the advantage of making it possible to bring together the most diverse effects both quantitative and qualitative into one simple concept. This concept, furthermore, has had a wide application to physiological processes. It may

be unique in its application to auxins only in this respect, that there is a large number of known active substances and that there are several types of effects which may be estimated by different methods. Furthermore, these methods are all relative, so that negative as well as positive values can be recorded in terms of distinct effects on growth.

The above scheme will account for the inhibition of growth by a relatively high concentration of a single active auxin on the assumption that in the presence of excess auxin some molecules will be attached to the enzyme system and others to the active groups of the substrate thus effectively blocking each other from reacting.

Competitive auxin action and the mechanism of bud inhibition.—The present scheme makes possible an extension of the "direct mechanism" of auxin action in bud inhibition presented by Thimann and Skoog (1934), Thimann (1937), and Skoog (1939). The term *direct* has been generally used to designate an effect exerted by the auxin in the inhibited bud as opposed to effects which may be produced on the bud indirectly as a result of influences of auxin on the utilization or translocation in the stem of nutrients and more or less specific growth factors toward or away from regions of active growth.

Some principal objections raised to the concept of *direct* auxin action in inhibition can now be met. One of these has been based on the fact that the application of very low auxin concentrations to decapitated plants or isolated buds will stimulate rather than inhibit bud development (Thimann, 1937; Went, 1939b; Skoog, 1939). This result can be explained as follows: In the period immediately after decapitation of the stem or removal of the buds, the free auxin content of the latter is known to be very low. Small additional amounts of auxin may, therefore, be absorbed by the available enzyme-substrate system without its saturation value for optimal growth being exceeded. Addition of low concentrations will hence lead to acceleration rather than inhibition of bud growth.

A second objection has been that on the basis of mass action one auxin cannot be considered to affect the formation or activity of another chemically different substance (as, for example, indoleacetic acid affects auxin A). This difficulty is eliminated when it is recognized that the relationship between the substances depends not on their being chemically identical but is determined by their ability to occupy the same position in a large molecular aggregate.

Another observation, hitherto unaccounted for, can be explained. This is the necessity for a continuous supply of auxin to a bud to effectively inhibit its growth. A total concentration much higher than that actually required for complete inhibition of growth when the supply is maintained at a constant rate is completely ineffective when supplied in such a manner that, for short intervals between applications, the concentration is allowed to fall to a very low level. It is clear that in these intervals growth of the bud will proceed at optimal rates and as the bud en-

larges, increasingly higher dosages will be required for inhibition of its growth.

It has been reported that the concentration of auxin in a bud cannot be a determining factor in the inhibition of bud development, as inhibited buds may contain either higher or lower total extractable amounts of auxin than growing buds (van Overbeek, 1938; Ferman, 1938). It must be considered, however, that conditions leading to inhibition are determined not by the concentration of *total extractable* auxin but by the relative concentrations of the *free* auxin, enzyme proteins and substrates, *i.e.*, by the equilibrium between auxin and other components of its reaction system. Now it becomes evident that organs or tissues may have very different auxin concentration requirements for growth, as for example roots and shoots, or adjacent tissue layers in the stem, and further that these requirements will vary for each organ in its successive stages of development. It follows, moreover, that in correlative inhibition, as in the inhibition of one developing bud by another, the auxin of the inhibited bud may be actually decreasing in quantity at the same time that it is becoming increasingly effective in inhibitory action, as the supply of substrate is being utilized by the more rapidly growing bud. As soon as the slightest difference in growth rates arises between two buds the auxin system will begin to act as an automatic device facilitating and gradually making effective a complete shift of growth from one bud to the other. This interpretation concedes the importance of factors other than auxin in correlative inhibition. Yet it emphasizes the special rôle of auxin as an immediate or direct regulatory agent. The controversial question whether auxin acts directly in the bud or indirectly in the stem becomes irrelevant. It is clear that inhibition can be and under experimental conditions has been accomplished by a sufficiently high concentration of hormone in the bud itself. If, however, at the same time, as in intact plants, auxin present in the stem causes acceleration of growth and utilization of substrate, then the concentration of auxin required in the lateral bud to cause inhibition of its growth will be greatly reduced.

Finally it has been suggested that the variety in types of growth responses obtained with different auxins in a single plant or with a single auxin in different plants precludes the possibility of a single general auxin reaction (Hitchcock and Zimmerman, 1938; Went, 1939a). From a consideration of auxin-apoenzyme-substrate equilibria, identical final growth responses can be expected only from identical cells treated alike with the same substance, no matter how simple may be the chemical reaction carried out by the auxin molecule itself. (Compare variability in behavior of plant dehydrogenases.) Therefore, the type of response will depend not on the auxin reaction but on the structural configuration of the auxin molecule and on the types of protein and substrate with which it is combined.

CONCLUSION.—Concepts which have been proposed to explain the action of auxins and the rela-

tive activities of different auxins are inadequate to account for effects obtained with mixtures of phenylbutyric and indoleacetic acids on the growth of *Avena* coleoptiles. A consideration of these effects and the evidence from recent literature on the action of auxins suggests rather a general scheme for auxin action essentially similar to that employed for the action of coenzymes. Briefly it contains the following points:

(1) The properties of auxin essential for its activity are (a) a structural configuration which will allow it to occupy specific positions in a larger molecular aggregate, and (b) a specific chemically reactive group.

(2) The relative activities of different auxins are determined by each of these requirements, though the two may be in part interrelated.

(3) Auxins with adequate structural configuration but with low chemical reactivity will compete with more active substances for available positions in the enzyme-substrate system and will thus block the action of the latter (examples: phenylbutyric acid, indolethyl amine, auxin-precursor from radish).

(4) Auxins with high reactivity but with less adequate structure will have low activity and will not function effectively as inhibitors (example: phenylacetic acid).

(5) High concentrations of a highly active auxin will also lead to growth inhibition by the effective blocking of one molecule by another from simultaneous contact with both enzyme and substrate.

The above scheme for the action of auxin is incomplete in details and may need modifications. Nevertheless, it covers the salient features of available experimental data. It permits the integration of the large variety of effects resulting from auxin activity in plants including interpretations of several hitherto unexplainable observations. It does so without ascribing qualitative differences to separate active substances and without introducing any peculiarities other than structural configuration to the auxins as a group. The main reason for its presentation, however, is that it offers a new approach for experiments on the function of auxin especially in relation to associated factors in the growth of plants.

SUMMARY

The effects on growth of indole-3-acetic and γ -phenylbutyric acids supplied separately and in mixtures have been studied by various standard methods in the *Avena* coleoptile.

The results show that phenylbutyric acid might possess slight auxin activity, but when it is administered in combination with indoleacetic acid, its main effect is to inhibit the activity of the latter. The extent of this inhibition is a function of the relative concentrations of the two substances and is, therefore, ascribed to competitive action between them.

The presence of phenylbutyric acid in solutions or plant tissues prevents quantitative estimations of their auxin activity (plant auxin or indoleacetic acid). Phenylbutyric acid cannot be effectively elimi-

nated for analytical purposes by ether extraction, diffusion in agar blocks or transport through plant tissues.

Phenylbutyric acid does not possess the properties implied in the definition of a "hemiauxin." The increase in response to indoleacetic acid elicited in the presence of phenylbutyric acid under certain conditions is attributed to an acid effect or a slight auxin activity of high concentrations of phenylbutyric acid and its "sparing action" especially on low concentrations of indoleacetic acid.

The results conform to the view that auxin acts as a coenzyme.

On this basis a scheme is presented for the action of auxins in stimulation and inhibition of growth.

This scheme affixes an important determinative rôle also to interactions between auxin and its

enzyme-substrate complex. It thus provides for the contribution of formative effects from the cell milieu and nutrients, and it avoids ascribing separate kinds of activity to different auxins to account for discrepancies in the types of response they may produce. Hence it emphasizes the immediate regulatory or correlative action of auxin in the growth process, and it accounts for the ability of auxin to accelerate or inhibit growth as a quantitative manifestation of a single function rather than as results of separate qualitative effects.

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DEVELOPMENT OF THE OVULE AND THE FORMATION OF THE SEED IN *PLANTAGO LANCEOLATA*¹

Geo. Olds Cooper

PLANTAGO LANCEOLATA L. (rib grass, ripple grass, English plantain) has been investigated by McCullagh (1934) and Tjebbes (1928) for the chromosome number, and by Souèges (1926) for polyembryony. The stages in microsporogenesis follow the usual history found in many of the angiosperms (Cooper 1935, 1938). Little detailed study of the development of the ovule prior to fertilization has been made. The development of the embryo of *Plantago* was studied by Souèges in 1923. The recent investigations of Cooper (1941) on *Phryma leptostachya* and Farley and Hutchinson (1941) on *Medicago* have shown the functional relationship of the vascular strand to the development of the endosperm and the embryo.

MATERIALS AND METHODS.—Floral spikes of *Plantago lanceolata* L. were gathered on the campus of the University of Wisconsin. They were fixed either in Carnoy's solution or in Karpenchenko's modification of Navashin's solution, embedded in paraffin, sectioned at 12–14 μ , and stained in Delafield's haematoxylin.

DEVELOPMENT OF THE OVULE.—The floral spike is at first capitate, later becoming cylindrical. Each flower bears a single pistil of two united carpels. Each carpel produces a single ovule, and the two ovules are borne on the axile placenta. The archesporial cell differentiates early in the hypodermal layer at the apex of the ovule. This cell is easily identified, since its cytoplasm is denser and the nucleus is larger than in the surrounding cells of the ovule (fig. 1). When the archesporial cell has become fully differentiated the single integument appears as a meristematic outgrowth from the chalazal cells immediately below the archesporial cell and grows upward and about the apex of the nucellus (fig. 2, 3). As the integument is forming, there is a more rapid growth of the ovule on the side away from the main axis and the ovule bends downward and around toward the base of the ovary. This bending continues as growth progresses, so that the ovule is anatropous at maturity. The nucellus consists of a single layer of cells around the archesporial cell except at the chalazal end. The integument becomes massive and a long micropyle leads to the nucellus (fig. 10, N) which is now deeply embedded within the ovule. The cells of the nucellus toward the micropylar end as the megagametophyte approaches maturity have elongated and flattened and, shortly after fertilization, disappear.

In the early development of the ovule the main vascular strand of the ovary extends into the axis part way to the base of the ovules (fig. 2). It differentiates further as the ovules increase in size and the

axis elongates. A branch from the main vascular strand penetrates the funiculus to the chalazal end of each ovule and is separated from the mature megagametophyte by several layers of parenchyma cells which function as conducting cells (fig. 10). These cells are undifferentiated and are uniform in their cytoplasmic content. However as the megagametophyte matures the adjacent conducting cells elongate slightly, and the cytoplasm becomes highly vacuolate (fig. 11). The conducting tissue nearer the apex of the vascular strand is made up of cells in active mitosis and elongation. They remain finely vacuolate until the ovule is almost mature. These conducting cells function in osmotic conduction from the vascular strand to the developing megagametophyte. The vascular strand in the later stages in the development of the ovule can be distinguished at the tip of the funiculus between the micropylar and chalazal haustoria. The funiculus appears narrower due to the active development of the micropylar and chalazal haustoria which have digested the parenchyma of the funiculus between the ovule and the placenta (fig. 19, 21). The remaining parenchyma cells of the funiculus have lost most of their cytoplasmic content prior to the formation of the abscission layer.

MEGASPOROGENESIS AND THE DEVELOPMENT OF THE MEGAGAMETOPHYTE.—The archesporial cell functions as the megaspore mother cell and enlarges and elongates while the integument is growing. The cytoplasm of this cell becomes finely vacuolate, and the nucleus enlarges during the early stages of meiosis. Six pairs of chromosomes are present at diakinesis (fig. 4). Likewise, at the heterotypic metaphase (fig. 5) and at the heterotypic anaphase six pairs of chromosomes are present (fig. 6). A linear tetrad results from the two meiotic divisions (fig. 7). The megaspore toward the chalazal end becomes the functional megaspore. The three megaspores at the micropylar end disintegrate before the megagametophyte is formed. The nucleus of the persisting megaspore divides mitotically so that a two-nucleate cell results. The two nuclei of this cell migrate apart, one going toward the micropylar end and the other toward the chalazal end (fig. 8). These nuclei divide again (fig. 9), two remaining at the chalazal end of the developing megagametophyte and two at the micropylar end. Cell walls appear following a third division of these nuclei and a typical seven-celled megagametophyte is formed consisting of three antipodals, two synergids, the egg cell and the large primary endosperm cell (fig. 12). The two antipodals at the chalazal end are elongated and lie side by side; the third is cubical and is located below the other two and adjacent to the primary endosperm mother cell. The synergids, obovate in shape with a deep staining granular cytoplasm, surround in part the egg cell. The egg is large and pear-shaped with a reticulate cytoplasm that is finally vacuolate about

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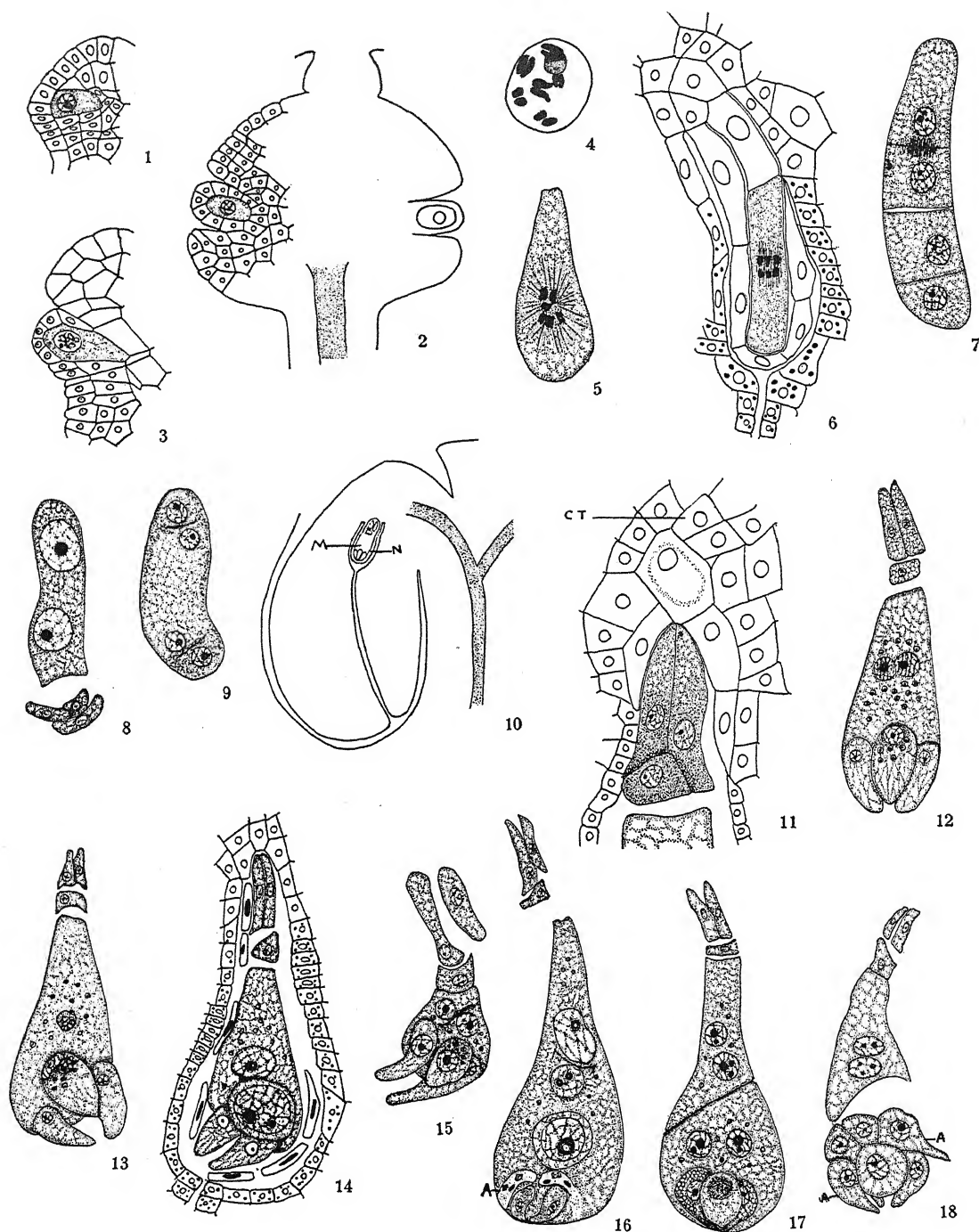


Fig. 1-18.—Fig. 1. Young ovule with the archesporial cell functioning as the megaspore mother cell. $\times 330$.—Fig. 2. Longitudinal section through ovules showing vascular strand. $\times 330$.—Fig. 3. Enlargement of the megaspore mother cell and early development of the integument. $\times 425$.—Fig. 4. Diakinesis; six pairs of chromosomes are present. $\times 1650$.—Fig. 5. Megaspore mother cell with nucleus at heterotypic metaphase. $\times 1000$.—Fig. 6. Nucleus of megaspore mother cell at heterotypic anaphase, conducting cells at chalazal region. $\times 800$.—Fig. 7. Tetrad of four megaspores. $\times 1000$.—Fig. 8. Two-nucleate megagametophyte and disintegrating cells at chalazal region. $\times 1000$.—Fig. 9. Four-nucleate megagametophyte. $\times 1000$.—Fig. 10. Longitudinal section through ovule with megagametophyte (M), nucellus (N), and vascular strand of funiculus. $\times 190$.—Fig. 11. Longitudinal view of chalazal end of megagametophyte showing three antipodals and conducting tissue (C.T.). $\times 800$.—Fig. 12. Mature megagametophyte. $\times 475$.—Fig. 13. Double fertilization. $\times 475$.—Fig. 14. Megagametophyte after fertilization showing zygote and 3n endosperm nucleus. Nucellar cells disintegrating. $\times 450$.—Fig. 15. First division of endosperm nucleus. $\times 450$.—Fig. 16. Presence of pollen tubes after fer-

the nucleus and coarsely vacuolate at its pointed end. The two polar nuclei fuse shortly after the formation of the megagametophyte. Starch grains are present in the egg and the primary endosperm mother cell only. An abundance of starch is present in the endothelial layer and adjoining cells of the integument at the micropylar end and to a lesser degree at the chalazal end about the megagametophyte.

FURTHER DEVELOPMENT OF THE OVULE.—The cells of the nucellus toward the micropylar end, which have elongated and separated from one another during the development of the megagametophyte, disappear shortly after fertilization (fig. 14). Both ovules of a given pistil mature in flowers at the base of the floral spike, whereas in late maturing flowers one ovule may become a seed while the other one, not being fertilized, may become aborted. Pollination in any particular flower occurs before the anthers of that flower mature. A high percentage of sterility is present in the pollen grains in the anthers. Several pollen tubes may enter a megagametophyte prior to fertilization and persist for a time after the formation of the zygote (fig. 16). The collapsed tube nucleus and the two shrunken male gamete cells can be seen within an inactive pollen tube (fig. 16, A).

FERTILIZATION AND EARLY DEVELOPMENT OF THE ENDOSPERM.—Double fertilization occurs; the nucleus of one male gamete unites with the nucleus of the egg, the other with the primary endosperm nucleus (fig. 13). The nuclei of the egg cell and the primary endosperm cell now show a marked increase in size (fig. 14). Following the first division of the endosperm nucleus (fig. 15, 16), a cell-plate is formed. The dividing wall lies diagonally across the primary endosperm cell (fig. 17). The nucleus of the chalazal cell divides again, remains binucleate, and the nuclei increase in size. The micropylar cell undergoes two divisions to form four cells that partly surround the zygote (fig. 18). The two outer cells are larger and conical (fig. 18, A); the two inner cells are more or less cubical. The two inner cells undergo further divisions and in time produce the endosperm proper, consisting of many thin-walled cells, except for the peripheral layer which has thick walls and dense cytoplasmic content. This layer retains its characteristic appearance throughout the further growth of the endosperm. The endosperm is at first spherical in shape, later becoming kidney-shaped. The antipodals meanwhile have disintegrated and the synergids which persisted for a time disappear.

DEVELOPMENT AND BEHAVIOR OF THE HAUSTORIA.—Haustoria are developed from specialized cells of the endosperm and aid in the conduction of food from the cells of the integument and nucellus to the developing embryo and endosperm proper. The lower cell in the two-celled endosperm stage functions as the chalazal haustorium. As it digests its way through the parenchyma of the funiculus to the placenta it elongates and becomes club-shaped at the growing

tip, with greatly enlarged nuclei. Continued growth of the chalazal haustorium in time separates the basal portion of the ovule from the placenta, except for the few cells about the vascular strand in the funiculus (fig. 19, 21). The two outer cells derived from the divisions of the micropylar cell of the two-celled endosperm are uninucleate in structure, rarely becoming binucleate and function as the micropylar haustoria. These micropylar haustoria are spreading in their growth and at their extremities produce pseudopodium-like outgrowths (fig. 21). The pseudopodial outgrowths apparently secrete substances which digest the parenchyma of the funiculus and the placenta so that in time the funiculus appears much reduced in size between the chalazal and micropylar haustoria. The cytoplasm of the haustoria is highly vacuolate and is denser at the tips of the pseudopodium-like outgrowths that are in contact with the cells of the integument. The presence of an abundance of starch in the cells of the integument adjacent to these haustoria indicates the presence of stored foods which may possibly stimulate their growth upon being digested. The middle lamellae of the cells of the integument are absorbed first, later the adjacent walls and finally the cellular content (fig. 22). Similar behavior of the mature haustoria was observed by Balicka-Iwanowska (1899) and Souèges (1926).

Branches from the micropylar and chalazal haustoria were observed penetrating and digesting the outer portion of the ovule adjacent to the developing endosperm. These branches differ slightly from the main haustoria in that they contain no nuclei and are highly vacuolate with concentrated amounts of cytoplasm at their tips (fig. 19, A). The growth of these haustorial branches throughout the chalazal end and the outer region of the integument likewise differ from the main haustoria in that, whenever a branch tip penetrates the integument, there seems to be pressure exerted, and the adjacent cells of the integument become flattened and elongated (fig. 20).

DEVELOPMENT OF THE SEED.—The division of the zygote to form the apical cell and the basal cell in the early development of the embryo is delayed until the endosperm with its haustoria is well differentiated (fig. 19). The apical cell of the embryo in a series of divisions forms the embryo proper, and the basal cell divides to form the suspensor. The course of development of the embryo is in accord with the description of Souèges (1923) for the species. As the hypocotyl and the cotyledons grow, the epicotyl is recognized as an opaque region at the apex of the embryo between the cotyledons. The developing embryo lies in a plane parallel to the longitudinal axis of the ovule (fig. 23). The embryo within the surrounding endosperm does not become very large and absorbs only a small amount of the endosperm. The endosperm however absorbs most of the surrounding integument as it increases in size, so that only a few cell layers

tilization. $\times 450$.—Fig. 17. Second division of endosperm nuclei. Cell at chalazal end functions as haustorium. $\times 450$.—Fig. 18. Chalazal haustorium with two nuclei; conical cells (A) become micropylar haustoria; cubical cells become the endosperm proper. $\times 450$.

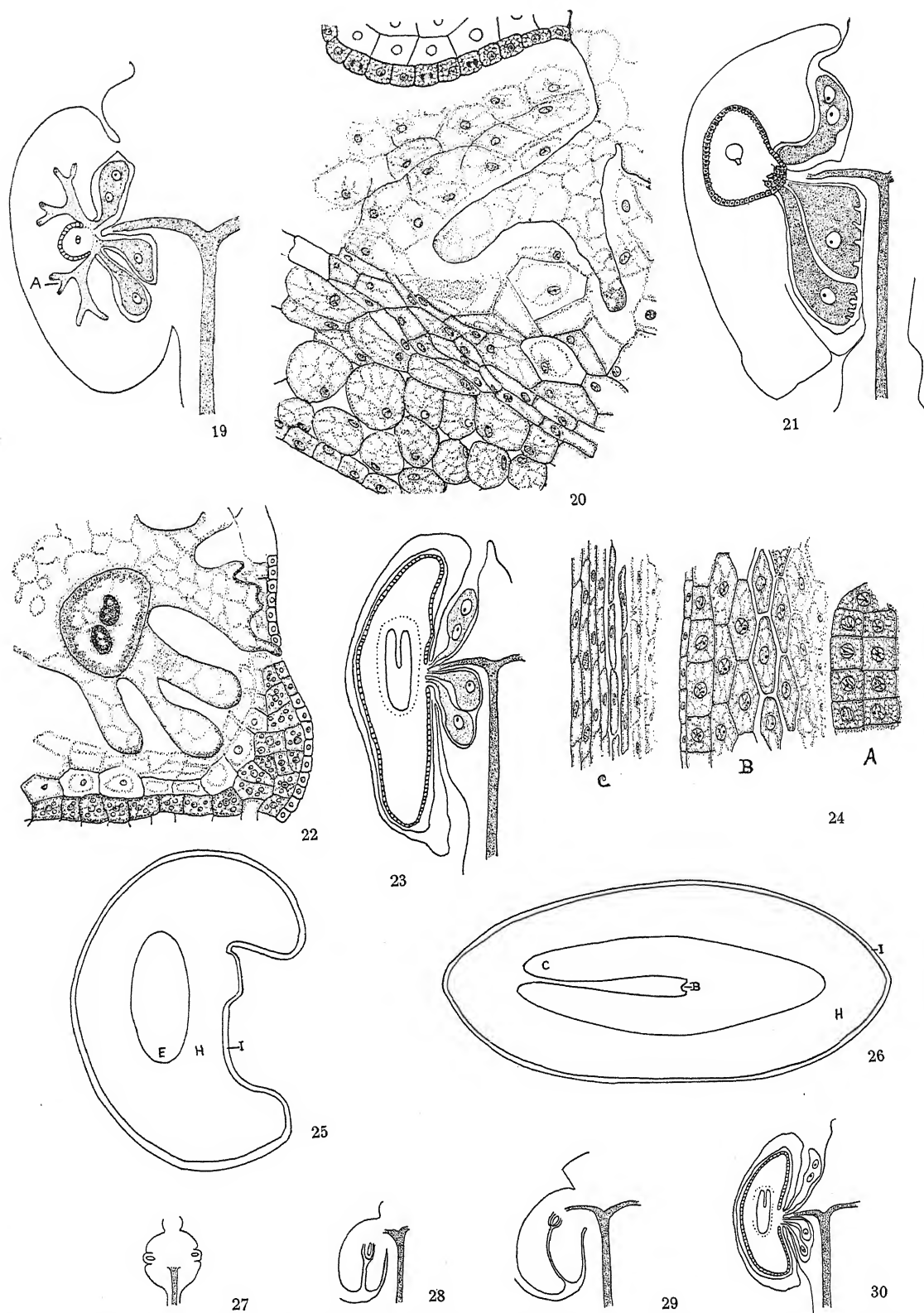


Fig. 19-30.—Fig. 19. Two-celled embryo surrounded by endosperm; developing chalazal and micropylar haustoria absorbing the parenchyma of funiculus toward the placenta. Branches from haustoria digesting integument. Vascular strand and conducting tissue in the funiculus. $\times 350$.—Fig. 20. Detail of region (A) of figure 19 showing the digestion

of the integument remain (fig. 24). At maturity the endosperm with its characteristic surface layer of cells occupies the major portion of the seed and completely surrounds the embryo. The remaining cells of the integument become much elongated in forming the seed coat, lose most of their cytoplasmic content and are papery at the time of seed dispersal (fig. 25, 26). The mature seed contains the embryo, embedded in an abundant endosperm, and a papery seed coat. Remains of the micropylar and chalazal haustoria are present in the locule between the seed and the axis of the ovule.

SUMMARY

Each carpel of the two-loculed pistil contains an ovule which is anatropous at maturity.

A single archesporial cell in the hypodermal layer of the ovule functions as the megaspore mother cell. A single massive integument is formed.

Six pairs of chromosomes are present at meiosis.

Four megaspores are formed in a linear row and the chalazal megaspore develops into an eight-nucleate, seven-celled megagametophyte; the other megaspores disintegrate. The cells of the nucellus begin to disintegrate at this stage.

A vascular strand is differentiated early in the funiculus to the chalazal end of the ovule and functions in conduction from the main vascular strand of the ovary to the conducting cells at the chalazal end of the megagametophyte.

Abundant starch is present in the integument about the megagametophyte and, to a lesser degree, in the primary endosperm cell and the egg.

Double fertilization occurs. The endosperm divides to form two cells; the chalazal cell becomes binucleate and functions as an haustorium. The micropylar cell divides to form four cells, two of which by a series of divisions form the endosperm proper; the remaining two function as the micropylar haustoria. The growth of these haustoria in time separates the ovule from the placenta, except for the vascular strand of the funiculus.

The developing endosperm is delineated by a definite row of cells that persist even in the mature seed.

The zygote does not divide until the endosperm has become many-celled.

The mature seed with its papery seed coat contains the embryo embedded within the endosperm.

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- of a portion of the integument by an haustorial branch. $\times 700$.—Fig. 21. Further growth of the haustoria, enlarging endosperm and developing embryo. $\times 60$.—Fig. 22. Detail of micropylar haustorium (fig. 21, A) penetrating integument toward axis. $\times 400$.—Fig. 23. Embryo digesting endosperm and the embryo absorbing the inner cells of the integument. Haustoria have almost separated ovule from placenta, as indicated by the absorption of the parenchyma of the funiculus. $\times 180$.—Fig. 24. Longitudinal section of ovule at cotyledonary level, (A) embryo, (B) endosperm, inner layer of cells being absorbed by embryo, (C) integument, inner layer of cells being absorbed by endosperm. $\times 500$.—Fig. 25. Cross section of mature seed, (E) embryo, (H) endosperm, (I) papery seed coat. $\times 35$.—Fig. 26. Longitudinal section of seed, (I) seed coat, (H) endosperm, (A) hypocotyl, (B) epicotyl, (C) cotyledon. $\times 35$.—Fig. 27-30. Diagrams to illustrate the relation of the vascular strand and the conducting tissue in the development of the anatropous ovule.—Fig. 27. The main vascular strand.—Fig. 28. The bending of the ovule and the branching of the vascular strand into the funiculus.—Fig. 29. The vascular strand within the funiculus to the conducting tissue at the chalazal end and the completed bending of the ovule.—Fig. 30. Vascular strand of the funiculus persisting after the haustoria have digested the parenchyma of the funiculus.

OBSERVATIONS ON THE GENUS *BLASTOCLADIELLA*¹

J. N. Couch and Alma J. Whiffen

THE GENUS *Blastocladiella*, discovered by Matthews in 1937, is a saprophytic aquatic phycomycete which in its thallus structure is intermediate between the simplest fungi, the Chytridiales, and some of the more complex forms such as *Blastocladia* or *Allomyces*. At present the genus is composed of the original species *B. simplex* Matthews and *B. variabilis* Harder & Sörgel. *B. simplex* produces zoosporangia and non-sexual resting sporangia and thus has a simple life cycle without any alternation of sporophytic and gametophytic asexual and sexual generations. In soil collections from South Carolina and Texas, we have isolated three new species of this rare genus. The two from South Carolina have life histories similar to *B. simplex*, but the one from Texas has a life cycle which differs from that of any other species in the genus.

ISOLATION AND DEVELOPMENT OF THE NEW SPECIES.—The two species from South Carolina were found on the same piece of boiled grass leaf, occurring there when the grass leaf was first examined, in the form of a few zoosporangia and smooth and warted-walled resting sporangia which might be either stalked or sessile. It was at first suspected that we had found the resting sporangia of *Clavochytridium stomophilum*. The two species were isolated in unifungal culture in the following way. Part of a leaf with zoosporangia and resting bodies was cut out and put in a Petri dish with a fresh piece of a boiled leaf of *Paspalum* grass and water. Twenty-four hours later on part of this piece of leaf there was what appeared to be a pure growth of the new chytrid. This part of the leaf was cut out and put on 3 per cent plain agar, and then, under a binocular microscope, eleven single zoosporangia were dissected out from the leaf tissue, each dragged to a fresh part of the agar, then cut out with a small block of agar and transferred to the surface of a new piece of leaf in a sterile dish. In some of these isolations and in the cultures descended from them, only resting sporangia with rough walls have appeared, and in others only the smooth-walled resting sporangia. It was thus established that the rough- and smooth-walled resting sporangia belonged to different species. The species with rough-walled resting sporangia is given the name *Blastocladiella aspersperma*, the smooth-walled one is *B. laevisperma*.

In these two species of *Blastocladiella* the development is much the same and appears to be quite similar to that of *B. simplex* as described by Matthews (1937).

The following observations have been written up from notes made on *B. laevisperma*. The zoospore encysts and sends out a delicate germ tube which branches and enlarges to become the rhizoidal system (fig. 16, 17, 18). The spore itself enlarges great-

ly to form the stalked or sessile zoosporangium or resting sporangium. In the development of the zoosporangia the protoplasm in the early stages shows the pale, whitish gleam characteristic of the chytrids, with minute vacuoles and numerous fat bodies of variable size. The fat bodies, however, are never so large as in such chytrid forms as *Rhizophidium*. As development proceeds the protoplasm becomes more conspicuously vacuolated and densely granular. Just before the zoosporangium reaches its mature size the numerous fat bodies break up into small particles and become evenly dispersed through the protoplasm (fig. 19, 20). Soon after the basal wall is formed, the tiny globules become arranged in a reticulate fashion (fig. 21). About this time one or more emergence papillae are formed and simultaneously the tiny globules collect into small groups, a group in the center of each spore origin (fig. 22). A few minutes later the outlines of the zoospores appear as rounded, clear areas each with several small globules (fig. 23). In some sporangia just after the spores are completely formed the spore mass near the center may start cyclic motion which increases in vigor until most of the spores have been discharged. In other sporangia there is no autonomous movement of the spores until after they emerge.

When the spores are ready to emerge, the hyaline cap of the papilla gelatinizes, and part of the spores push out into this hyaline substance to form a spherical mass. The spores are retained in this ball for only a few seconds, for the gelatinous membrane quickly breaks and the spores swim away. The remaining spores emerge one or several at a time depending upon the size of the emergence pore. As seen under a dark field the spores swim smoothly in a hardly perceptible spiral path rotating clockwise on their axes, the cilium showing alternating single and double images. All quiet spores showed a conspicuous tail piece on the cilium under the dark field.

In the development of the resting sporangia in *B. aspersperma*, *B. laevisperma*, and *B. simplex* we have been unable to detect any fusions of zoospores. Such fusions may occur, for we have not followed the development of a single unfused zoospore into a resting body. Thalli developed from single zoospore strains produce both zoosporangia and resting bodies, which indicates the absence of sexual strains and the absence of an alternation of sexual and asexual generations. In the early stages of development the thalli of resting sporangia cannot be differentiated from those of zoosporangia. After they have reached mature size, however, the protoplasts of the resting sporangia shrink from the original enclosing membrane and secrete a new, thick, double-layered wall which becomes the wall of the resting sporangium. The resting sporangia are capable of germination only after a rest period of several weeks, and, though we have tried a number of experiments to shorten this period, we have so far been unable to

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do so. We have found, furthermore, that prolonged drying of the resting sporangia of *B. aspersperma* and *B. laevisperma* on pieces of leaves in Petri dishes kills them; whereas similar treatment or even drying for as long as a year has no effect on the vitality of the resting sporangia of *B. simplex* or the new species from Texas. The process of germination is essentially alike in all species of *Blastocladiella* which we have studied.

In *Blastocladiella variabilis*, Harder and Sörgel (1938) have described a fungus with a much more complex life cycle than is found in the "simplex" group. In that species there is a distinct alternation of generations, the sporophyte generation consisting of two kinds of plants: a zoosporangial and a resting sporangial plant. The former gives rise to zoospores which germinate to produce either another zoosporangial thallus or a resting sporangial thallus, but when the latter germinates it gives rise to two kinds of zoospores, one of which grows into the plus or female gametangial thallus; the other growing into the minus or male gametangial thallus. The female gametangium is colorless, the male faintly reddish, and they form uniciliated gametes which fuse in pairs to form a zygote that grows either into a zoosporangium or a resting sporangium. The life cycle of this organism and of *Sphaerocladia* (see below) represent the most complex life cycles yet described in the lower fungi and, perhaps, in all fungi save the rusts, for here are found a distinct alternation of sexual and asexual generations and, furthermore, the sexual plants are heterothallic.

A third type of life cycle is illustrated by *Blastocladiella cystogena*, so called because of its cyst-forming habit. This heretofore undescribed fungus was isolated in April, 1940, from soil from Texas, but was not critically examined until after our studies on the two species from South Carolina were well under way. Indeed, the form from Texas was passed over by us as *B. simplex*, and it was only when we wished to compare that species with the ones from South Carolina that Miss Whiffen discovered that, when the resting sporangia of the form from Texas germinated, the zoospores encysted in an irregular mass, the contents of each cyst giving rise to four uniciliated zoospores. Further studies showed that these uniciliated zoospores fused in pairs to form a biciliated zygote which develops into a resting body. It is very likely that the plant body here is diploid and that only the gametes are haploid. Any definite conclusion, however, as to when reduction division occurs must await the outcome of detailed cytological studies now being made by Miss Hendricks in our laboratory.

In the development of this fungus we shall concern ourselves here only with the germination of the resting sporangia and the formation and fusion of the gametes.

The mature resting sporangia vary from colorless and thin-walled to thick-walled brown bodies. Some are capable of germinating immediately after their formation, while others seem to require a rest period.

Resting sporangia dried on a piece of leaf in a Petri dish were still capable of germination after a year. One may hasten the germination of resting sporangia by drying them for forty-eight hours or longer and then adding water. Pouring off the water from old cultures and adding fresh water will bring on germinations. Abundant germinations have been obtained at room temperature (18° to 24°). The first sign of germination is the appearance of several narrow fissures in the brownish outer wall through which the gray protoplasm in the sporangium can be seen. (fig. 1, 2, 49, 50). Due to the continued swelling of the contents of the sporangium, the fissures widen and open into each other thus making an irregular tear in the outer wall. Through this opening one or two papillae grow out, after which the swarmers are delimited in the sporangium (fig. 1-3). In some sporangia the swarmers or zoospores show active movement before emerging, while in others they are inactive before reaching the outside. The zoospores emerge as in other species of *Blastocladiella* (fig. 4, 5). The end of the papilla gelatinizes, and some of the zoospores push outward, forming a spherical mass within a gelatinous sac. This gelatinous envelop quickly dissolves, and the remaining spores left in the sporangium emerge. The spores, though furnished with cilia, do not as a rule swim away as they emerge. They may swim for a short distance settling in the same field, but usually they stick together in one or more irregular heaps or twisted rope-like masses, their cilia waving for a few minutes before encystment (fig. 6). The cysts are spherical and usually of uniform size and contain hyaline protoplasm with a number of small yellowish globules (fig. 6, 7). The period of encystment lasts from thirty minutes to an hour.

About fifteen minutes after encystment a papilla is formed on each cyst, and shortly thereafter the protoplasm in each one is divided into four parts, each of which becomes a gamete (fig. 7, 58). It is likely that during gametogenesis a meiotic nuclear division occurs. Almost immediately after cleavage is complete the gametes begin to emerge. The first one to emerge pushes slowly through the papilla thus creating an opening, and the other three follow slowly one at a time. Sometimes one gamete may take as long as a minute to flow through the narrow pore. The four gametes from a cyst usually remain clustered for a few seconds or minutes at the mouth of the cyst, their cilia jerking feebly (fig. 7, 58). Each gamete is uniciliated and contains one nucleus with a conspicuous and characteristic body forming a cap over the part of the nucleus away from the cilium. Since there is considerable difference of opinion as to the nature of this body, we are calling it simply the nuclear cap. To the side of the nucleus and nuclear cap is a clump of several shiny, yellowish fat globules. The anterior end of the gamete is hyaline except for a few granules (fig. 59, a). It has been stated above that each cyst gives rise to four gametes, and this is the rule with rare exceptions, where, perhaps due to incomplete cleavage, a cyst may give rise to

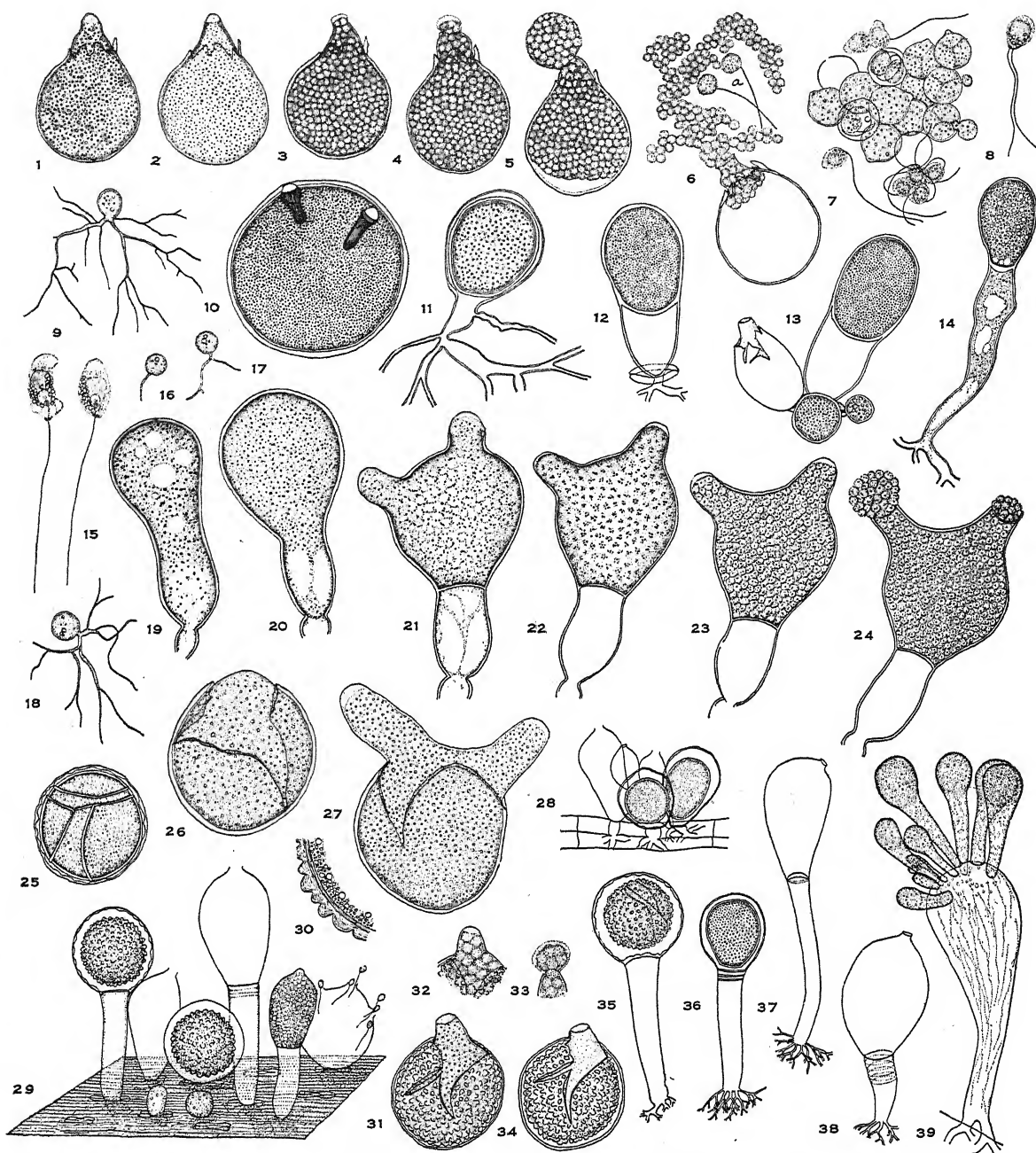


Fig. 1-39. Fig. 1-14. *Blastocladiella cystogena*.—Fig. 1-6. Germination of resting body, $\times 200$.—Fig. 6a. Uniflagellate zoospores just after being discharged from resting sporangium, $\times 390$.—Fig. 7. Germination cysts in the process of forming and discharging gametes, $\times 560$.—Fig. 8. Biflagellate zygote, $\times 560$.—Fig. 9. Young thallus developing in an agar culture, $\times 200$.—Fig. 10. Mature resting body showing peculiar thickening in the wall, $\times 200$.—Fig. 11. Mature thallus grown on hemp seed, $\times 200$.—Fig. 12. Stalked resting body on a grass leaf, $\times 200$.—Fig. 13. A group of germinated, mature, and young resting bodies protruding from a leaf stoma, $\times 200$.—Fig. 14. An immature stalked resting body from a hemp seed culture, $\times 200$.—Fig. 15-28. *Blastocladiella laevisperma*.—Fig. 15. An amoeboid and a swimming zoospore, $\times 930$.—Fig. 16-18. Stages in germination of zoospore, $\times 200$.—Fig. 19-24. A series of developmental stages of a zoosporangium, $\times 350$.—Fig. 19. 3:00 P.M.—Fig. 20. 8:00 P.M.—Fig. 21. 10:00 P.M.—Fig. 22. 10:30 P.M.—Fig. 23. 10:40 P.M.—Fig. 24. 10:45 P.M.—Fig. 25. An unstalked resting body showing several ridges on the wall, $\times 245$.—Fig. 26 and 27. Two stages in the germination of the resting body, $\times 390$.—Fig. 28. A group of empty zoosporangia and resting bodies on a grass leaf, $\times 190$.—Fig. 29-35. *Blastocladiella aspersperma*.—Fig. 29. Habit sketch, $\times 190$.—Fig. 30. Section of wall of resting body, $\times 760$.—Fig. 31-34. Stages in the germination of the resting body, $\times 390$.—Fig. 35. A stalked resting body, $\times 190$.—Fig. 36-39. *Blastocladiella laevisperma*.—Fig. 36. A stalked resting body showing successive rings formed in the stalk at the base of the resting body, $\times 190$.—Fig. 37-38. Empty zoosporangia, $\times 120$.—Fig. 39. A number of small young thalli parasitizing a large thallus, $\times 240$.

two biciliate structures. There is a remarkable simultaneousness about the emergence of the gametes from the same clump of cysts, so that, only a few minutes after the first have come out, the whole mass is seething with gametes.

Observations on fusing gametes have been made under a water immersion lens with a cover slip over the material, the presence of which did not seem at all to interfere with their activity. The cover glass, however, should be supported to prevent its weight from crushing the cysts. This support may be fur-

with it. Sometimes it may be slow to fuse with any one of the tetrad, the whole mass of five gametes gliding over one another for one or two minutes before fusion is effected. After cytoplasmic fusion has occurred it usually takes several seconds for the zygote to become organized for swimming. If the gametes have fused laterally with both cilia pointing in the same direction, this reorganization is relatively rapid. The two nuclear caps come in contact and flow together like two drops of oil; the two cilia come to lie parallel and very close together, perhaps

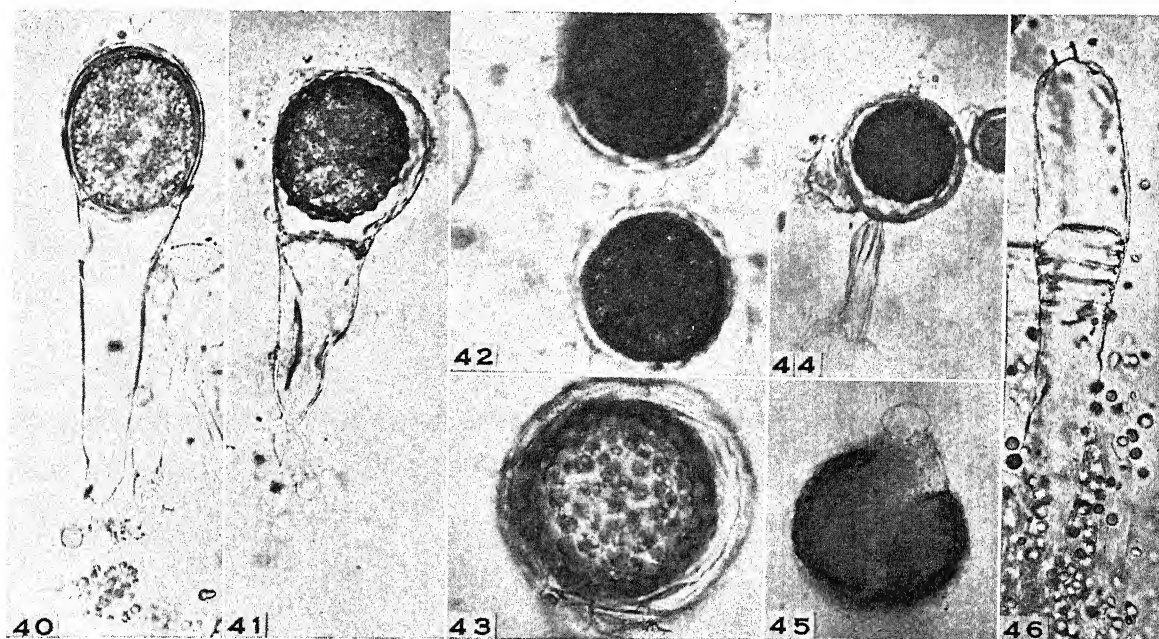
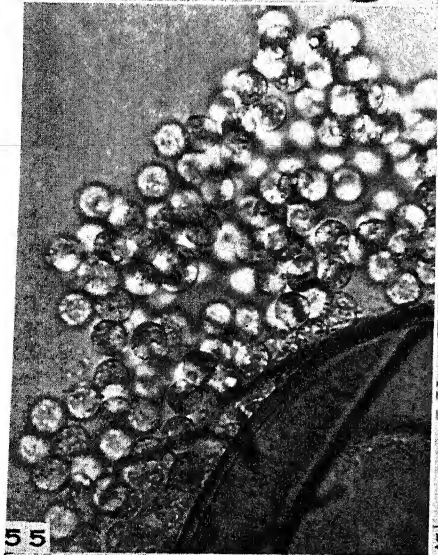
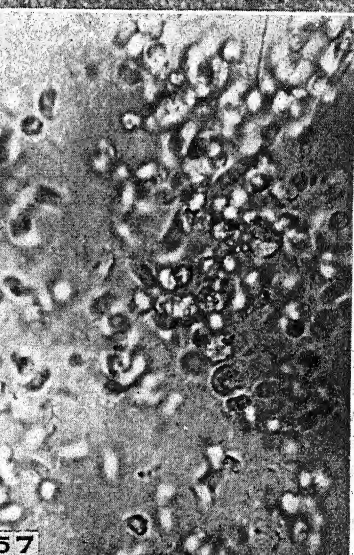
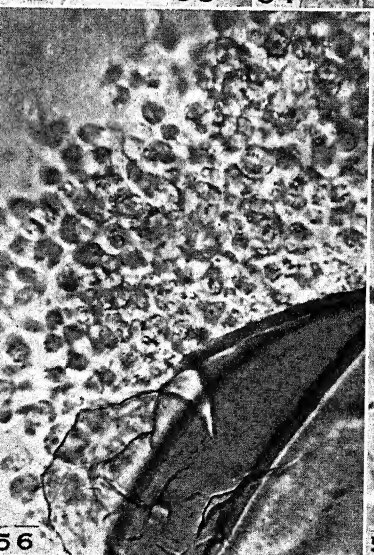
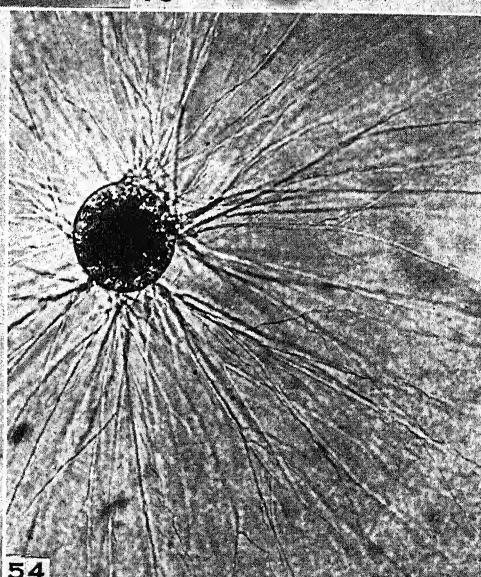
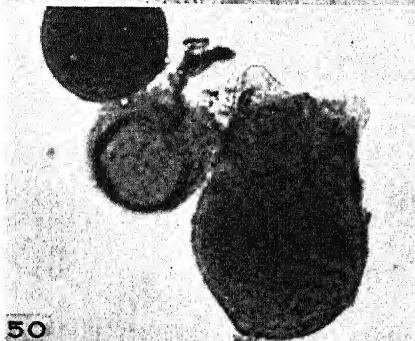
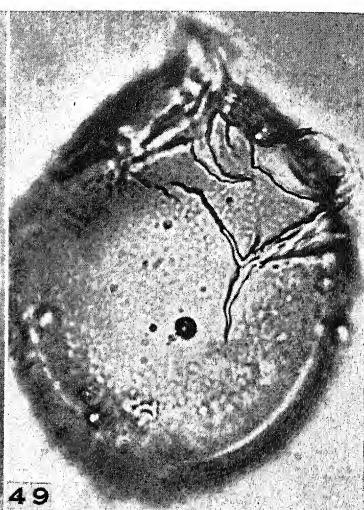
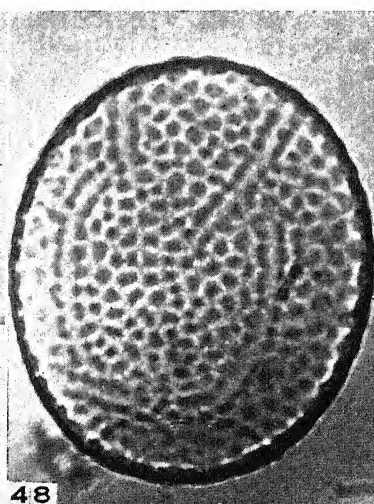
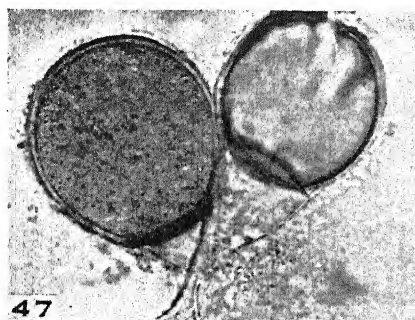


Fig. 40-46. Photomicrographs of *B. laevisperma* and *B. asperosperma*.—Fig. 40. Stalked resting body of *B. laevisperma*, $\times 400$.—Fig. 41. Stalked resting body of *B. asperosperma*, $\times 400$.—Fig. 42. Unstalked resting body of *B. laevisperma* grown on agar, $\times 430$.—Fig. 43. Unstalked resting body of *B. asperosperma* grown on a grass leaf, $\times 400$.—Fig. 44. Resting body of *B. asperosperma* showing constriction of rhizoid at base of the resting body, $\times 430$.—Fig. 45. Germinating resting body of *B. asperosperma*, $\times 400$.—Fig. 46. Empty zoosporangium of *B. laevisperma*, $\times 400$.

nished by keeping ample water under the cover slip and by mounting several extra resting sporangia with the ones about to discharge. When the gametes emerge, they may remain clustered at the cyst for a few seconds or minutes, after which they may break away and creep over the other gametes and cysts until fusion with a compatible partner occurs. Fusion usually occurs so quickly and with so little movement that one is likely to overlook the event (fig. 59). One sees two gametes side by side, and suddenly in their place appears a biciliated zygote. Fusions occur most frequently between gametes lying side by side with both cilia pointed in the same direction, but fusion may start from any part of the membrane. Gametes from the same cyst have not been observed to fuse with each other, but a gamete from a different cyst may swim or creep up to the cluster (fig. 59) and fuse immediately with the first gamete it touches, or it may move on to another and fuse

actually in contact; the nuclei fuse, though this cannot be clearly observed in living material; and the numerous yellowish fat globules come together in one compact clump, though they do not fuse. However, if the gametes have begun to fuse with their cilia not in the same direction it may take a minute or two and violent struggling before the cilia and the internal parts become properly oriented (fig. 61, a-d). In the process of fusion the two gametes show about an equal amount of activity, though sometimes one may be more active than the other or the two may alternate in brief periods of activity. Not infrequently two violently struggling gametes apparently in the act of fusing may be approached by a third gamete which immediately quietly fuses with one of the partners, the zygote swimming away and the unfused gamete creeping off to try its fortune elsewhere. Sometimes while the gametes are gliding over each other one may send out a pseudopodium which,



playing against the membrane of the opposite gamete, makes a connection, and then after a considerable struggle fusion is completed (fig. 62, a-k). Two gametes may become connected in this way and yet not fuse, for sometimes one of the gametes may jerk so violently as to break the connection and swim away (fig. 60). Again a third gamete may appear and quickly fuse with one of the two after which the zygote becomes free.

After fusion is complete the zygote suddenly gives several violent lunges to free itself from gametes, cysts or other entanglements and then swims away. The swimming zygotes were observed under the dark field, and it was found that the two cilia were so closely applied to each other as to appear as one, and, moreover, that they functioned as one in propelling the zygote (fig. 59e, 62k). The cilia do not fuse, however, for when a zygote stops swimming for a few seconds, the two cilia may separate from each other along their entire lengths. This behavior seemed rather unusual to us, for in abnormally biciliate spores of the chytrids, as in *Rhizophlyctis rosea*, the two cilia have never been observed to act as one. We have also found that the two cilia on the zygote of *Allomyces javanicus* functioned as one, just as in the zygote of *B. cystogena*. In the chytrids there is sometimes a fusion of motile cells, as in *Pringsheimiella dioica*, but it is possible that only when a true sexual fusion occurs do the two cilia become associated and act as one.

Fusion stages have also been observed in stained material. Excellent preparations can be made by fixing material in water on the slide for thirty seconds in fumes of 1 per cent osmic acid, and then staining with very dilute gentian violet. The violet should be so dilute that when the drop is held over white paper it is only faintly colored. If such a preparation is examined immediately under a water immersion lens, one can find various stages of nuclear fusions (fig. 63a-c). By the time the two cilia have come so close as to appear as one, the two nuclei have fused. The two cilia are as a rule in this position on the living zygote before it swims away, and one may, therefore, conclude that at this stage in living material the nuclei have fused.

TAXONOMY OF THE GENUS.—With the discovery of the three new species of *Blastocladiella* and particularly *B. cystogena*, the need for an extension of the limits of the genus is quite obvious. At the same time the question arises as to the taxonomic position of the two recently described genera, *Clavochytridium* and *Sphaerocladia*.

Clavochytridium (Cox, 1939) is certainly much closer to *Blastocladiella* than was thought when the former was first described. Indeed except for their larger size and usually swollen base, the zoosporangial thalli of *Cl. stomophilum* can hardly be distinguished from stalked zoosporangia of *B. aspersperma* and *B. laevisperma*. The zoospores also are alike in structure but differ slightly in behavior during discharge, a distinction which in view of the similarities seems hardly of generic value. We are, therefore, transferring *Cl. stomophilum* to the genus *Blastocladiella*.

The genus *Sphaerocladia* which has a life cycle very much like that of *B. variabilis* is separated from *Blastocladiella* by the spherical shape and the presence of a "seitenkörper" in the zoospore of the former. Stüben (1939) grew his plants on flies and on various types of nutrient agar. When grown on agar under a cover glass, stalked forms were produced. Under the culture conditions used by Stüben, we doubt if the absence of a stalk is of sufficient significance to justify separation from *Blastocladiella*. On agar and on leaves *B. simplex* may or may not have a stalk. On agar *B. cystogena*, *B. stomophilum* and *B. laevisperma* are never stalked, and the rhizoids may arise from any point on the surface of the zoosporangium or resting sporangium. On leaves *B. stomophilum* is usually stalked, *B. cystogena* is usually without a stalk or with a very inconspicuous short one and *B. laevisperma* and *B. aspersperma* are as frequently sessile as stalked. When grown on hemp seed in water, the stalked forms predominate in *B. simplex*, *B. cystogena*, *B. aspersperma*, and *B. laevisperma*.

Another basis for the separation of *Sphaerocladia* from *Blastocladiella* is in the presence of a side body (seitenkörper) in the zoospore of the former. In the living zoospores of *B. simplex*, *B. laevisperma* and *B. aspersperma* we are unable to find with certainty any structures which would be interpreted as Stüben's side body. However, when the zoospores of these species are killed with the fumes of osmic acid and weakly stained with an aqueous solution of gentian violet and studied immediately without drying, a very conspicuous curved body is visible. In *B. laevisperma* and *B. aspersperma* this body extends from near the rhizoplast over the nucleus and nuclear cap usually almost to the opposite end of the spore, varying somewhat in size in different spores (fig. 65, 66). The nuclear cap is deep purple or almost black, while the side body is much less deeply stained and appears to be hollow or vesicular in structure (fig. 63-66, s.b.) In *B. simplex* the side

Fig. 47-57.—Fig. 47, 49-57. Photomicrographs of *B. cystogena*.—Fig. 48. Photomicrograph of *B. simplex*.—Fig. 47. An ungerminated and a germinated resting body, $\times 400$.—Fig. 48. Resting body of *B. simplex* showing markings on outer surface of wall, $\times 600$.—Fig. 49. Germinated resting body of *B. cystogena* showing faint areolations on the wall, $\times 430$.—Fig. 50. A germinating resting body with two exit papillae $\times 150$.—Fig. 51. Spores encysting near the resting body from which they are being discharged, $\times 70$.—Fig. 52. A stalked resting body from a hemp seed culture, $\times 150$.—Fig. 53. A zygote which has been killed and stained to show the position of the two cilia, $\times 930$.—Fig. 54. An immature resting body growing on agar, $\times 100$.—Fig. 55-57. Successive stages in the germination of the cysts from one resting body, $\times 650$.—Fig. 55. Exit papillae have formed on the cysts.—Fig. 56. The contents of the cysts are divided up into four parts.—Fig. 57. Gametes are being discharged from the cysts. Note empty cysts in the lower left hand corner.

body is shorter and thicker than in the other species but has the same vesicular appearance. A particularly good view of the side body and indeed of the entire contents is shown in the spore in figure 64 in which the parts were considerably swollen and the plasma membrane had begun to disintegrate. The side body was much enlarged, purplish and clearly

Blastocladiella, as well as the two genera *Clavochytridium* and *Sphaerocladia*.

BLASTOCLADIELLA Matthews 1937, em. Harder & Sörgel 1938, 1939

Rhopalomyces Harder & Sörgel 1938

Clavochytridium Couch & Cox, in Cox 1939

Sphaerocladia Stüben 1939

Thallus microscopic, unicellular, consisting of a single extramatrical, tubular, pyriform or globose part anchored to the substratum by a branched rhizoidal system; extramatrical part giving rise to a stalked or sessile, thin-walled zoosporangium, resting sporangium, or gametangium. Thin-walled zoosporangia usually present, globose to cylindrical with one to several discharge papillae. Zoospores posteriorly uniciliate, with a nuclear cap over the anterior part of the nucleus and with several laterally placed fat or lipid globules. Thick-walled resting sporangia usually present, enclosed in the old thallus membrane; wall of resting sporangium composed of two distinct layers: the outer brownish, thick, usually sculptured layer, and the inner, hyaline, thin, smooth layer; outer layer coarsely or minutely areolate, warted or smooth or smooth except for one or several ridges. Resting sporangia germinating by the cracking of the exospore wall into two or several irregular pieces after which the hyaline inner part sends out one or more papillae through which zoospores emerge to form zoosporangia and/or resting sporangia, or to grow into thallose gametangia, or to encyst, the cysts acting as gametangia and germinating to release four gametes which fuse in pairs to form a zygote. Cell walls not giving a cellulose reaction with chloriodide of zinc.

On plant or animal substrata in fresh water habitats or on soil subject to flooding.

KEY TO THE SPECIES OF *BLASTOCLADIELLA*.

A. Short life cycle, only one generation

1. Both zoosporangia and resting sporangia present

- a. Thalli more than 500 μ tall; resting sporangia distinctly areolate *B. simplex*
- b. Thalli less than 500 μ tall

- i. Resting sporangia warted *B. aspersperma*
- ii. Resting sporangia smooth *B. laevisperma*

2. Only zoosporangia present

- a. Thalli frequently up to 300 μ tall and usually stalked on leaves *B. stomophilum*

B. Long life cycle, two equal generations, asexual and sexual

- 1. Sporangia and gametangia usually stalked; male gametangium orange *B. variabilis*

- 2. Sporangia and gametangia usually sessile; both gametangia colorless *B. Stübenii*

C. Short life cycle, only resting sporangia and cysts which act as gametangia formed *B. cystogena*

BLASTOCLADIELLA aspersperma n. sp.²—Thalli clavati vel globosi, basi cum rhizoideis; thalli duabus formis, similibus magnitudine et figura, altera zoosporangia, altera sporangia perdurantia parienti;

² The Latin descriptions were prepared by Alma Holand Beers.

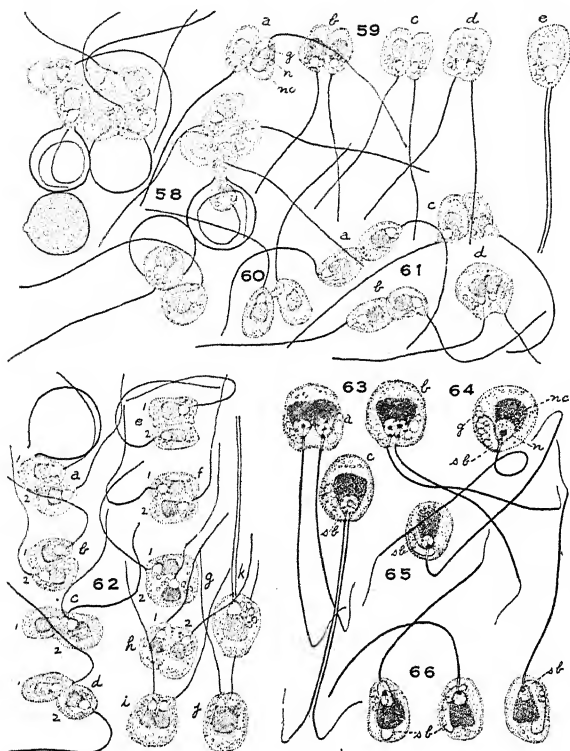


Fig. 58-66. Cysts, gametes, zygotes, and spores.—Fig. 58-63. *Blastocladiella cystogena*. Living gametes in various stages of emergence from cysts.—Fig. 59 a-e. Stages in fusion of a pair of gametes. n.c., nuclear cap; n., nucleus; g., lipid granules; v., vacuole.—Fig. 60. Two gametes connected by pseudopodium.—Fig. 61 a-d. Two gametes fusing at anterior end.—Fig. 62 a-k. Stages in fusion of a pair of living gametes.—Fig. 63 a-c. Gametes killed and stained to show nuclear fusion.—Fig. 64. *B. simplex*. n., nucleus; n.c., nuclear cap; s.b., side body; g., lipid granules.—Fig. 65. *B. laevisperma*.—Fig. 66. *B. aspersperma*. All $\times 1,050$.

vesicular. Adhering to the side body were the darkened fat bodies. There seemed to be an intimate association between the rhizoplast and the side body. Indeed it seemed that the cilium was attached both to the side body and the rhizoplast. With the discovery of side bodies in the zoospores of the type species of *Blastocladiella*, and three other species in addition, the final distinction between *Blastocladiella* and *Sphaerocladia* falls down and *Sphaerocladia variabilis* must be transferred to *Blastocladiella*. We suggest the name *Blastocladiella Stübenii*.

The following generic description and key to the species are drawn up to include all known species of

zoosporangiiis 25–100 μ diam. vel usque ad 88 \times 155 μ ; stipite nullo vel stipite usque ad 30 \times 150 μ . Zoosporis uniflagellatis, 3.6–4.6 \times 6–7 μ . Sporangiiis perdurantibus capsam non plene explentibus, globosis vel subcylindricis, fere 14–80 μ diam. (globosis); pariete flavo-brunneo, distincte verrucoso.

Thalli consisting of extramatrical stalked or sessile zoosporangia and resting sporangia anchored to the substratum by rhizoids; single or caespitose. Zoosporangial thalli spherical, ovoid, pyriform or nearly cylindrical; sporangia spherical, pyriform, ovoid, sub-cylindrical or irregular; 25–100 μ thick when spherical, up to 88 \times 155 μ when ovoid or pyriform; stalk when present varying from a small basal part to a long tube 30 \times 150 μ , usually thickest just below the sporangium, frequently with one to several rings just below sporangium. Sporangia with one to several (up to 11) emergence papillae. Zoospores usually becoming active in sporangium before emergence. The first spores emerging in a spherical vesicle formed from the gelatinized tip. After the vesicle bursts the spores remaining in the sporangium emerge slowly and swim away immediately upon reaching the outside. Zoospores 3.6–4.6 \times 6–7 μ . Resting sporangial thalli similar in shape, size and early development to the zoosporangial thalli; resting sporangia not completely filling the case in which they are formed; spherical, ovoid, pyriform or sub-cylindrical; outer wall yellow brown and distinctly warted; 14–80 μ thick on grass when spherical, somewhat larger on hemp seed and spherical to oval in shape. Germinating after several weeks' rest to form zoospores as in zoosporangia.

On pieces of boiled *Paspalum* grass leaves from soil collected in roadside ditch by Philip Couch on way to Middleton Gardens, South Carolina, June 15, 1941. This species is named *B. aspersperma* because of the rough walled resting spore.

BLASTOCLADIELLA laevisperma n. sp.—Thalli clavati vel globosi, basi cum rhizoideis; thalli duabus formis, similibus magnitudine et figura, altera zoosporangia, altera sporangia perdurantia parienti; zoosporangiiis 25–117 μ diam.; stipite nullo vel stipite usque ad 26 \times 105 μ . Zoosporis uniflagellatis, 3.8–4.6 \times 6–6.5 μ . Sporangiiis perdurantibus globosis vel subcylindricis, raro duobus sporangiiis perdurantibus in eodem thallo formati, fere vel plene capsam explentibus; pariete rubro-brunneo, laevi praeter lineas elevatas paucas.

Zoosporangial and resting sporangial thalli much as in *B. aspersperma* except resting sporangia usually fill the case and have a smooth wall except for a few ridges. Zoosporangia usually sessile on leaves and stalked on hemp seed; 25–117 μ when spherical; stalk up to 26 \times 105 μ . Zoospores 3.8–4.6 \times 6–6.5 μ when active, 5–5.4 μ when rounded up. Resting sporangial case as a rule stalked on hemp seed but usually sessile on leaves; rarely two resting sporangia may be formed on the same stalk; spherical, ovoid, pyriform or sub-cylindrical, sometimes constricted in the middle, at times lobed; 25–140 μ thick when spherical, up to 63 \times 95 μ

when ovoid; stalk 30 \times 155 μ , broadest at the top; resting sporangia usually completely filling the case and conforming to its shape, up to 126 μ thick when spherical; wall 1.5–2.8 μ thick, reddish brown or pale dull yellow, smooth except for one or several conspicuous ridges. Germinating after a few weeks' rest to form zoospores which are similar to those from zoosporangia.

In the same soil collection with *B. aspersperma*. The name *B. laevisperma* is given because of the smooth-walled resting sporangium.

The two species may be distinguished by differences in the resting sporangia. In *B. laevisperma* the resting sporangia are reddish brown, smooth walled and usually fill the case, while in *B. aspersperma* the resting sporangia are yellowish brown, with a coarsely warted wall and only partly fill the case.

BLASTOCLADIELLA cystogena n. sp.—Thalli nulli zoosporangia parientes; thalli sporangia perdurantia stipitati vel sessiles, basi cum rhizoideis, parientes. Sporangiiis perdurantibus globosis vel subglobosis, 16–342 diam. (globosis) vel 37–142 \times 39–564 μ ; pariete duplici, parte exteriori laevi vel minute areolata, prope hyalina vel aureo-brunnea; ut in genere germinantibus. Zoosporis uniflagellatis, extemplo in sporas immotas (cystae) massa irregulari transformati. Sporis immotis (gametangia) 8.2–10.2 μ diam., globosis. Omni spora immota quatuor cellulas sexuales uniflagellatas parienti, quae binae conjunctae zygosporas biflagellatas formant.

Zoosporangial thalli lacking in life cycle. Resting sporangial thalli when on leaves usually extramatrical, spherical or pyriform and sessile or with a very small and inconspicuous stalk, frequently intramatrical on leaves and then subspherical ovoid or lobed; on hemp seed usually tubular, the case that encloses the resting sporangium globose to subcylindrical. Rhizoids well developed, attached basally if sporangium is extramatrical, or attached to several places on the sides if intramatrical. Resting sporangia when spherical 16–342 μ thick, when subspherical 37–142 \times 35–209 μ ; stalk when present very variable in size, 30–116 \times 39–564 μ ; usually filling the case in which it is formed; wall two-layered, very faintly areolate, 1.5–6.1 μ thick, nearly hyaline to dull yellow or orange brown. Germination as in the genus. Zoospores uniciliate when discharged, encysting almost immediately near the sporangial mouth in an irregular mass. Encysted spores (gametangia) 8.2–10.2 μ thick, spherical. After one to two hours each cyst gives rise to four unciliated gametes which emerge through a minute pore to fuse in pairs near the empty cysts; gametes 4.1 \times 4.9 μ . Zygotes biciliated, 6 \times 8 μ , swimming for an hour or more before germinating to form resting sporangia.

On pieces of boiled *Paspalum* grass leaves from soil collected by Philip Couch March 20, 1940, at Laredo, Texas. This species is named *B. cystogena* because of the occurrence of an encystment stage when the zoospores emerge from the resting sporangia.

The above species is readily distinguished from all other species of *Blastocladiella* by the absence of a thin wall zoosporangial stage and by the life cycle with cysts and isoplanogametes.

DISCUSSION.—Miss Matthews (1937) in her original description of *Blastocladiella simplex* emphasized its close relationship to the Blastocladales, a viewpoint the correctness of which has been corroborated by the researches of Harder and Sörgel (1938) and our own. In the genus *Blastocladia*, the type of life cycle present is largely unknown except in the one species *B. Pringsheimii*, which has recently been studied in detail by Miss Blackwell (1940). This has the short life cycle as in *Blastocladiella simplex*. In *Allomyces* the life cycle types are much better known than in *Blastocladia*.

Emerson (1938, 1939, 1941) has shown that there are three distinct types of life cycles in *Allomyces*: (1) *Euallomyces* has the long cycle with sporophytic and gametophytic generations; (2) *Brachyallomyces* has the short cycle with only the zoosporangial and resting spore stage; and (3) *Cystogenes* has a short cycle with a cyst stage. There is a striking similarity between two of the types of life cycles found in *Allomyces* and in *Blastocladiella*, a fact recognized by Emerson (1941). *B. variabilis* and *B. Stübenii* have life cycles of the *Euallomyces* type, as found in *A. arbusculus*; *B. simplex*, *B. aspersperma*, and *B. laevisperma* have life cycles of the *Brachyallomyces* types, as seen in *A. anomalus*. In addition to these two types we are adding a third which in certain respects is markedly similar to the *Cystogenes* type in *Allomyces*. In *A. cystogena* the same thallus produces thin-walled zoosporangia and pitted-walled resting sporangia. According to Emerson the latter germinate to produce biciliated zoospores which emerge to encyst at the mouth of the sporangium. The cysts later give rise to zoospores, usually four from each cyst, which swim for some time and then grow into plants like the parent. According to Emerson (1938, 1939, 1941) there is no gametic fusion in the cystogenes group of *Allomyces* though he has suggested that sexual fusions may take place during the germination of the resistant sporangia. In a paper which has just appeared McCranie (1942) describes observations which are at complete variance with those of Emerson. McCranie, using Emerson's Burma 1B isolate, reports that the spores from the resistant sporangia were without cilia encysting in a group near the sporangial opening. Immediately or after a rest period of variable duration the cysts give rise to secondary swarmers which become semi-amoeboid and copulate in pairs to form a biciliate zygote. In view of these strikingly different results, it is obvious that more work must be done on the cystogenes group of *Allomyces*. In *Blastocladiella cystogena* the thallus produces only resting sporangia and unciliated spores, which after encystment give rise to isogamous planogametes. These fuse in pairs to form a biciliate zygote. If we accept Emerson's description of the life cycle of *Allomyces cystogenus*, it is only in the production of

cysts that *Blastocladiella cystogena* resembles that species. If McCranie is right, however, then the parallel between the two is most striking indeed; for then it is highly probable that the vegetative thalli in both fungi are diploid.

It has been thought by all students of this group of fungi that *Blastocladiella* was a connecting link between the Blastocladales and the Chytridiales. If we believe in the ascending evolution of these forms, there can be little doubt that *Blastocladiella* represents the most primitive type of the Blastocladales. However, in spite of the close similarity of thallus shape in *Blastocladiella* to some of the Chytrids, there is no chytrid genus which appears to be very closely related to *Blastocladiella*. Of all the Chytrids known, perhaps *Cylindrochytrium* (Karling, 1941) resembles *Blastocladiella* most closely in thallus structure. This relationship, however, is not very close, for in *Cylindrochytrium* the zoospores and resting bodies are typically chytridiaceous, while this is not true in *Blastocladiella*. The zoospores of *Rhizophlyctis rosea* contain several small refractive globules as do those in *Blastocladiella*, but in cell wall and resting sporangial structure the two differ markedly. In the Blastocladales the resting sporangia may or may not completely fill the case; however, in all species the wall of the resting sporangium is readily separable from the outer case, as indicated by the freeing of the resting sporangium from the case in *Allomyces*, while in the Chytridiales the resting sporangium usually completely fills this wall. The resting bodies of the chytrids, with few exceptions, germinate by the formation of a zoosporangium outside of the old resting body, while in *Blastocladiella* and its relatives the resting body is transformed directly into a zoosporangium. In *Rozella*, *Pringsheimiella* and *Olpidium* the resting body germinates much as in *Blastocladiella*.

Although there seems to be no single chytrid genus that is closely related to *Blastocladiella*, there are certain basic morphological features shared commonly by the Chytridiales and the Blastocladales. All chytrids and members of the Blastocladales have the whiplash type of cilium (Couch, 1941) and are posteriorly ciliated even when there are two cilia. One structural feature which was supposed to be peculiar to the Blastocladales is the nuclear cap in the spore. Karling (1937) and Hillegas (1940), however, have described nuclear caps in several genera of the Chytridiales, and it is very likely that such structures are of common occurrence in this order. Thus it seems that the motile cells are basically alike in the two orders except for the usual presence of only one fat globule in the zoospore of the chytrids, while in the Blastocladales there are several fat bodies. It may be that the side body of Stüben is peculiar to the Blastocladales. Before this can be determined, however, a comparative study of the internal structure of the zoospores in the Chytridiales must be made.

The nuclear cap is one of the most striking characteristics of the *Blastocladia* type of spore and will

probably be found in all true chytrids. This cap is undoubtedly of phylogenetic importance, and it is of significance that Debaisieux (1920) has described such a structure in *Coelomycidium simulii*. This latter organism is, in the opinion of Debaisieux, closely related to the Chytrids and is put by him in this order. We are of the opinion, however, that *Coelomycidium*, while perhaps not a true Chytrid, is more closely related to the Chytridiales than to the Plasmodiophorales or the Protozoa.

SUMMARY

In soil collections from South Carolina and Texas three new species of *Blastocladiella* have been isolated. Two of these, *B. aspersperma* and *B. laevisperma*, have short life cycles without any alternation of generations and without sexuality like *B. simplex*. The other, *B. cystogena*, has a new type of life

cycle. It lacks the typical zoosporangial stage, but forms thick-walled, brownish-orange resting bodies, which after maturity may be brought to germination. When this happens, a large mass of unciliated zoospores emerge, which, instead of swimming, encyst in an irregular heap. After about an hour, the contents of each cyst divide into four unciliated gametes which emerge and fuse in pairs, no fusion occurring between gametes from the same cyst. The zygotes swim for some time, the two cilia acting as one, and then come to rest and germinate to form resting bodies. It is likely that reduction occurs when the gametes are formed, and, if so, the thallus of this species is diploid. A new description of the genus is given to include *B. cystogena*, *Clavochytridium stomophilum* and *Sphaerocladia variabilis*.

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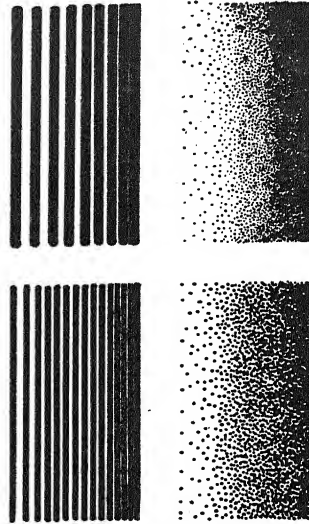
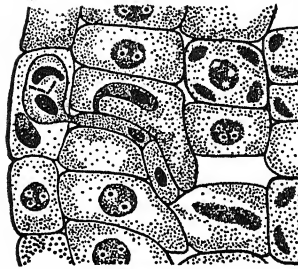
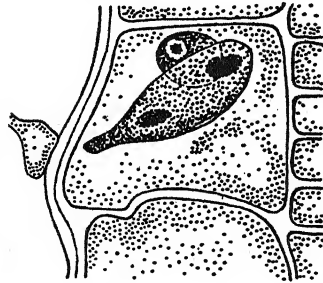
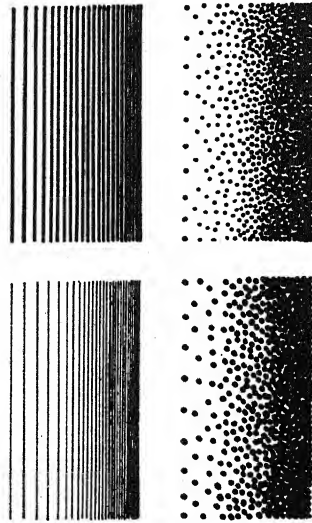
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DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

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Note that thin black lines hold up fairly well in reduction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. Keep the shading rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

NOTES ON THE ORIGIN OF ADVENTITIOUS ROOTS IN THE NATIVE ONTARIO CONIFERS ¹

M. W. Bannan

MUCH STUDY has been given of late to the propagation of conifers by cuttings (Deuber, 1940; Deuber and Farrar, 1940; Farrar and Grace, 1942; Griffith, 1940; Thimann and Delisle, 1942, and others). Considerable work has been done on determining the most satisfactory media for setting out, the best time of year for collection, the most suitable kinds of cuttings to use, and the concentration and types of hormones which are most effective in promoting root development. The anatomy of root formation, on the other hand, has received only slight attention, and in the present paper it is proposed to deal with this aspect of the problem. It should be emphasized, however, that the descriptions which follow apply only to layered material collected from the field, and not to specimens which have been experimentally treated in the laboratory.

TAXUS.—The prostrate *Taxus canadensis* Marsh is common in southern Ontario, less so in northern parts of the province. It sometimes grows in the open, but more often is found on the forest floor in the more deeply shaded woods. The decumbent branches are frequently covered by débris for much

of their length, and these buried parts are generally rooted.

As a rule the roots are not scattered singly along the branch but are grouped near the bases of lateral branches. Sometimes the lateral branches have rotted away (fig. 1); in other cases they are living (fig. 2) and may be almost as large as the principal branch. The roots in each group are sometimes even-aged, transverse sections showing all to have arisen in the same year; whereas in other instances various members in the group were initiated in different years. Some roots are short-lived; others attain considerable size with several rings of secondary xylem.

The relationships between adventitious roots and the xylem tissues of the branch are illustrated from transverse sections in figures 9–11. The first-formed cells of the tissue associated with root development are parenchyma cells which, as shown in the figures, are arranged in radial rows conterminous to the preceding tracheids. Later this parenchymatous tissue increases in width, sometimes gradually (fig. 10), sometimes abruptly (fig. 9), and in turn is succeeded by the root tissues proper.

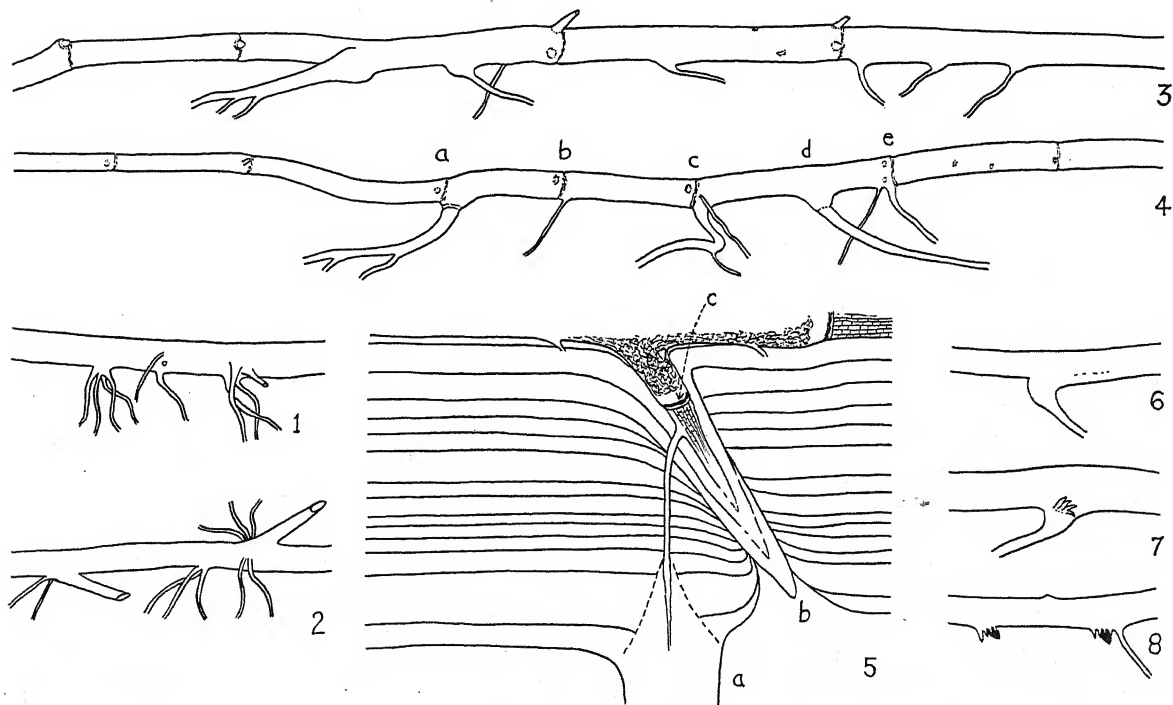
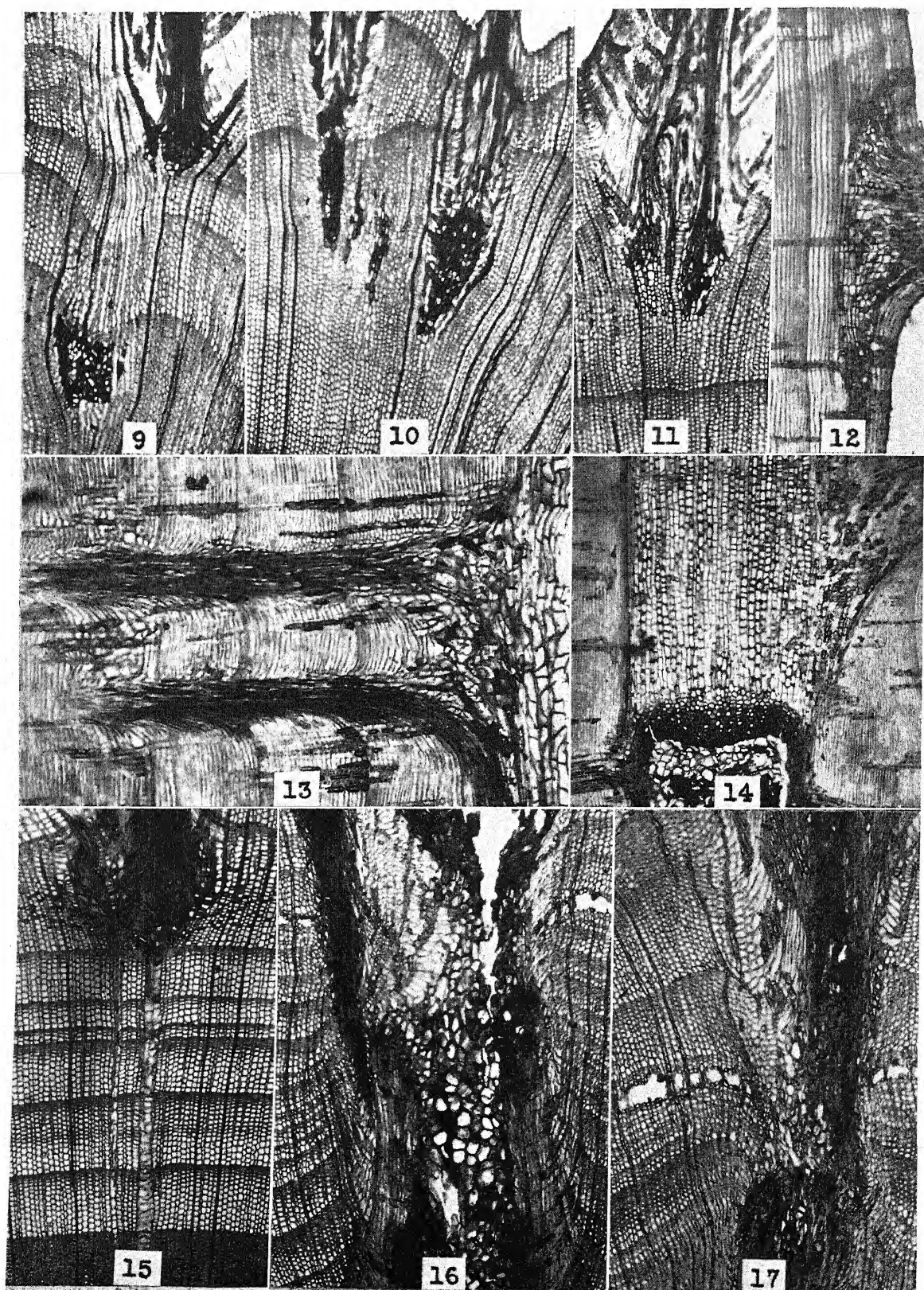


Fig. 1–8.—Fig. 1, 2. Portions of layered branches of *Taxus canadensis* showing distribution of roots.—Fig. 3. Same of *Abies balsamea*.—Fig. 4. Same of *Picea mariana*.—Fig. 5. Radial section of branch of *Picea mariana* showing relationship between root (a) and embedded lateral branch (b).—Fig. 6–8. Portions of rooted branches of *Larix laricina*.

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Examination of serial tangential sections reveals the first-formed parenchyma cells to be in vertical series resembling xylem parenchyma. Generally the fusiform outline of each series of cells is clearly discernible. Further, in radial sections it may be seen that the terminal cells of each series are in line with the upper and lower ends of the preceding tracheids in the same radial row. Such relationships indicate that the parenchyma cells and the conterminous tracheids were derived from the same fusiform initials. These parenchyma cells are soon succeeded by others which tend to decrease in height, to lengthen in the horizontal direction, and to become organized into radial rows (fig. 12).

The relationships described above indicate that the roots originate from sectors of the cambium consisting of variable numbers of fusiform initials and the intervening ray initials. In some instances it appeared that only one or two fusiform initials took part in root formation (fig. 10), but in approximately one-half the sixty-six cases studied the tangential extent of the cambium consumed ranged from four to six cells and in a few instances was as extensive as ten or more cells (fig. 11). The change from a vertical to a radial arrangement in the cells, and the simultaneous vertical contraction and tangential broadening of the parenchymatous tissue in the first-formed parts of the root tissue (fig. 12), suggest that the fusiform initials were subdivided into shorter units, the most centrally located of which increased in number and formed the root meristem.

In some cases a mass of parenchymatous tissue was laid down without root development, as illustrated in the lower part of figure 9. Serial tangential sections of such areas disclosed at some points a succession of cell types in which tracheids were followed by xylem parenchyma with the latter succeeded by tracheids again. Here it would seem that certain derivatives of the fusiform cambial cells were segmented to form the parenchyma cells, but the cambial cells themselves were not subdivided and maintained their shape more or less unaltered through successive divisions. At other points some of the fusiform elements (tracheids and xylem parenchyma) were followed by short cells which in successive sections tended to decrease in number and alter in shape, some being pinched out, others becoming more elongate again. In these cases there was apparently a subdivision of some of the fusiform cambial cells into shorter units. Subsequently certain of these, after brief periods of activity, ceased dividing; whereas others underwent gradual elongation to acquire a fusiform outline again.

Nearly all the parenchyma cells in the first-formed tissues associated with root development had relatively thin walls with little or no lignification. Only

a few sporadic cells were somewhat thicker walled and slightly lignified. As the cells became shorter and tended to lengthen in the radial direction tracheary cells appeared among the parenchyma. The first of these were usually more or less rhomboidal or rectangular in outline with numerous bordered pits on all walls. Toward the exterior the shape became more definitely radially elongated, and the radial and horizontal walls particularly had crowded pits exhibiting various mixtures of alternate and opposite arrangements. These tracheids gradually gave way to the primary xylem in the root proper.

THUJA AND JUNIPERUS.—The development of adventitious roots occurs frequently in *Thuja occidentalis* L., *Juniperus horizontalis* Moench, *J. communis* var. *depressa* Pursh, and when the opportunity offers, in *J. virginiana* L. As described in previous articles (Bannan, 1941 and 1942) the roots connect with peculiar xylem rays which differ from the ordinary rays in their larger size and, especially in *Thuja*, in the more frequent occurrence of tracheary cells. The association between root and ray is illustrated in figure 15. The root meristems apparently are organized from the cambial or adjoining phloem portions of the unusual rays. There was no evidence of fusiform cambial cells being involved as in *Taxus*.

ABIES.—Layering in *Abies balsamea* (L.) Mill. has been reported by Chittenden (1905), Cooper (1911), and Fuller (1913). The layering was noted in trees growing in habitats ranging from deep forest to exposed rocky ridges and mountain tops, but was best developed in the latter types of site. Rooted material examined by the writer was collected from trees growing in the forest and in nearby open areas, such as abandoned fields, which were being invaded. The collections were made in Northumberland County and Parry Sound and Muskoka Districts.

Rooting seemed to occur whenever the branches were buried. In some cases, particularly in the forest, the terminal portions of the branches turned upright and bore lateral branches in whorls so as to resemble small trees. In the open the distal parts of the rooted branches were usually bent only slightly upward and rarely became upright. The roots were scattered without pattern along the lower side of the buried parts of the branches, no aggregation in any particular part of the year's growth being evident (fig. 3).

In all the specimens examined the anatomical relationships between the roots and branches were similar. As shown in figure 13 each root connected inwardly with a radial sheet of parenchymatous tissue which resembled a branch gap and was located above and parallel to a leaf trace.

Fig. 9-17.—Fig. 9-11. *Taxus canadensis*, transverse sections of branch wood showing origin of adventitious roots.—Fig. 12. *Taxus canadensis*, radial section showing origin of root.—Fig. 13. *Abies balsamea*, radial section of branch wood illustrating root trace and subtending leaf trace.—Fig. 14. *Picea Abies*, radial section of branch showing medullary crown and gap associated with dormant bud.—Fig. 15. *Thuja occidentalis*, transverse section of branch wood illustrating unusual ray and connection with adventitious root.—Fig. 16, 17. *Larix laricina*, transverse sections of branch wood showing adventitious roots arising in vicinity of embedded xylem portions of dwarf branches.—Fig. 14, $\times 28$; others $\times 49$.

On the exterior of the distal portions of the branches very small dormant buds were recognized in the axils of leaves at intervals from the tip down as far as the eighth year, below which point they tended to become embedded in and indistinguishable from the bark. Associated with each dormant bud was a connection of parenchymatous tissue extending to the pith and located directly above a subtending leaf trace. The anatomical relationships resembled those described for the roots. It seems clear that the roots arose from dormant axillary buds which were stimulated into activity on being buried and gave rise to roots rather than lateral branches. Although Chittenden (1905) did not cite the evidence upon which his opinion was based, his statement that rooting in layered balsam proceeds from dormant buds is apparently confirmed.

The portion of the root gap nearest the pith was usually composed of parenchyma cells of varied rounded, rectangular or angular shapes. The walls were generally moderately thick and lignified, but here and there were very thin-walled ghost-like cells containing crystals. Oddly, in most cases there was no protoxylem associated with the part of the gap nearest the pith. Only in a few instances did small strands of spiral or scalariform tracheids extend along the bottom or sides of the gap and connect with the protoxylem in the main branch. Farther out the gap tended to contract vertically and to widen tangentially, the shape changing from narrowly oval in tangential section to round. The parenchyma cells became more regularly arranged in horizontal (radial) rows and tended to be definitely elongated in that direction. Beginning usually in the second or third annual rings there was a noticeable increase in the number of tracheids associated with the parenchyma cells. These tracheids were generally relatively short, and many were curved or bent so as to resemble the so-called "arm-pit" tracheids found along the under side of leaf traces. The tracheids as a rule were pitted, but had reticulate or irregular spiral thickenings on the inner side of the walls. Toward the exterior these tracheids became more numerous and ultimately more or less completely ensheathed the parenchymatous tissue in the center. In the succeeding growth rings there was no appreciable increase in size until the root was initiated, whereupon secondary tissues were added. In the specimens studied the time of root formation varied from the eighth to the seventeenth years.

As described above a striking feature of the root gaps was the absence or feeble development of accompanying protoxylem tissue. Traces were indistinguishable or of small size, consisting at the most of only a few spiral or scalariform tracheids. Similar conditions obtained in the case of the smallest lateral branches, but the larger branches, especially those at the upper end of the year's growth, generally had wide gaps with numerous traces extending along the base and sides of the gap.

PICEA.—Layering in *Picea mariana* (Mill.) BSP. has been reported by Cooper (1911) both for trees

growing in the forest and in bogs, and by Fuller (1913) for trees in exposed rocky areas. Cooper reported the habit for *P. glauca* (Moench) Voss on nearly bare rocks, but Fuller was unable to find many instances of rooting in this species.

Most of the material studied by the writer was black spruce collected from bogs in the Parry Sound District. The more widely spaced trees had branches to the ground, the lowest branches usually being bent so that their median portions were buried in the sphagnum or débris. These buried parts were generally rooted. In most cases the distal portions of the branches turned only slightly upward; seldom did they become upright to produce the "hen and chickens" appearance described by Fuller (1913). Much more difficulty was experienced in finding rooted branches of *Picea glauca*. A wide area of Renfrew and Lanark Counties in eastern Ontario was scouted, but very few instances of rooting were discovered. Although many trees had branches to the base, these were rarely covered by soil and hence little opportunity was provided for rooting.

In *Picea mariana*, unlike *Abies balsamea*, the roots were generally located in close proximity to the terminal bud scars (fig. 4), a distribution noted by Cooper (1931) in *Picea sitchensis*. As shown in figure 4 at (a), (b), and (c) most of the roots were situated immediately above or distal to the terminal bud scars; less often they were short distances below, as at (e); and in only a few instances were they distributed elsewhere along the branch as at (d). Sectioning of the wood showed that the roots located close above the terminal bud scars arose from the main branch, whereas the majority of those below, as at (e) in figure 4, originated not from the principal branch but rather from embedded basal portions of lateral branches whose distal parts had been cast off. This association is illustrated in figure 5. Here the root (a) arose from a lateral branch (b), connecting with it close above a crown of thick-walled cells (c) in the pith. This crown is a layer of cells separating the mature part of the old growth from the young tissues of the bud which later gave rise to the lateral branch. The lateral branch died, the distal part was exfoliated, and only the basal portion remained and gradually was surrounded by the secondary xylem of the principal branch. The relationship between the root and the lateral branch was thus similar to that between such roots as shown at (a), (b), and (c) in figure 4 and the main branch. In all these cases the roots were initiated in the basal part of the year's growth.

Lewis and Dowding (1924) have shown for *Picea glauca* that the position of the terminal bud is marked in the pith by a crown of thick-walled cells. Above the crown the pith cells are in regular longitudinal rows; below they are disordered and often more or less broken down to form a cavity. Similar terminal diaphragms of thick-walled cells were observed in *P. mariana* and *P. Abies* (fig. 14). In nearly all layered branches studied the roots connected inwardly with parenchymatous tissue extend-

ing to the pith close above such medullary crowns, the association resembling that illustrated in figure 14. The relationships between the root gaps and crowns were generally similar, whether the roots arose from the principal or from the basal parts of embedded lateral branches. In only a few instances, where the roots originated in the central part of the year's growth some distance from the terminal bud scars, were the root gaps subtended by leaf traces as in *Abies*.

Examination of the exterior of the distal portions of branches revealed the presence of small dormant buds close above the terminal bud scars. In radial sections it was seen that these buds connected with parenchymatous gaps located directly above the medullary crown (fig. 14). The dormant buds thus had the same anatomical relationships as the roots on the layered branches.

Although the roots in *Picea*, as in *Abies*, evidently arose from dormant buds,² the distribution of the roots was quite different in the two genera. In *Abies* the roots were distributed along the branch and, as a rule, were not associated with the terminal buds; in *Picea* they were usually located immediately above the terminal bud scars. In *Abies* the root gaps were subtended by leaf traces; in *Picea* the gaps were nearly always found close above medullary crowns, rarely some distance away and associated with leaf traces as in *Abies*.

As in *Abies* the portion of the root gap adjoining the pith was composed of variously shaped parenchyma cells. Most of these were thick-walled, but a few were thin-walled and contained crystals. Toward the exterior the cells became more uniformly elongated in the radial direction, and their arrangement became more compact. In some cases no protoxylem tracheids could be discerned accompanying the part of the gap nearest the pith. In other instances small traces extended along the bottom or sides of the gap and connected with the main system in the branch. From the second or third year outward a considerable development of "arm-pit" tracheids took place, and eventually these more or less ensheathed the centrally located parenchyma cells. So long as the bud remained dormant there was no appreciable increase in the diameter of the trace complex, but with development of the root secondary xylem was added.

In most instances the buds which developed into lateral branches had larger and more numerous primary vascular strands than the dormant buds. In general the largest and best vascularized buds were located toward the upper end of each year's growth, and these buds usually produced the most vigorous lateral branches. Downward the buds tended to decrease in size and to have less extensive primary vascular systems. A noticeable reduction in size of the lateral branches developing from these buds was

²In some hormone-treated cuttings of *Picea Abies* kindly sent the writer by Dr. N. H. Grace most of the roots arose at the cut end of the branches, the origin differing from that observed in the uninjured layered branches described above.

also apparent. Toward the base of the year's growth the buds were poorly vascularized, many having no discernible protoxylem, others only very small traces. These buds generally remained dormant, unless stimulated to growth by some unusual circumstances such as the burial of that part of the branch, whereupon they produced roots.

LARIX.—*Larix laricina* (Du Roi) Koch growing in bogs along with black spruce has been reported by Cooper (1911) as layering abundantly. In most of the swamps visited by the writer, however, the lower branches of the larch trees were dead and unrooted. The only rooted specimens discovered were growing in dune areas near the shores of lakes. At Presqu'Île on Lake Ontario a few widely spaced trees whose lower branches had become covered by shifting sand were layered, and similar material was obtained for the writer by Dr. Kathleen and Mr. Donald Hull from Inverhuron Beach on Lake Huron.

Nearly all the roots on the layered branches arose in close proximity to dead dwarf (leaf-bearing) branches. Sometimes no trace of the dwarf shoot remained on the exterior (fig. 6) but here sectioning of the branch revealed the basal portion of a dwarf branch buried inside. The number of roots associated with each dwarf branch varied greatly. Sometimes there were one or two moderately large functioning roots and a circlet of small poorly-developed roots which failed to grow appreciably in length (fig. 7 and 8); occasionally there were only a few rudimentary roots (fig. 8); and in other cases only one or two large roots were formed (fig. 6).

The total number of rooted specimens found was not great, and the relationships between the roots and the dwarf branches were not precisely similar. In some instances the roots were connected with the end or sides of the xylem core of the buried part of the dwarf branch (fig. 16 and 17). In another case the dwarf branch apparently was broken off relatively early and the roots arose from the wound calus covering the injury. In yet another instance the roots grew out from the secondary xylem of the main branch surrounding the dwarf branch. Since none of the specimens studied showed the earliest stages of development, one can only speculate on the nature of the cells which formed the root meristems. It seems most probable that the latter were organized from the cambium or recently formed undifferentiated tissue adjoining the dwarf branch.

PINUS AND TSUGA.—The writer is aware of only one reference in the literature to layering of pine in America. Lutz (1939) reported a single instance in *Pinus Strobus*. Rooting of hemlock branches has been described for *Tsuga Mertensiana* by Lutz (1930), and for both western species, *Tsuga Mertensiana* and *T. heterophylla*, by Cooper (1931). In Ontario the writer was unable to find layering in any of the native species of pine or hemlock (*Pinus Strobus* L., *P. resinosa* Ait., *P. Banksiana* Lamb., or *Tsuga canadensis* (L.) Carr.). The habit of the trees was usually such as to make layering impos-

sible, but even in pine plantations where the lower branches were sometimes in contact with the ground no roots were discovered.

SUMMARY

Certain anatomical features of the origin of adventitious roots in the native Ontario conifers were studied from layered material collected in different parts of the province.

In *Taxus canadensis* the roots arose from sectors of the cambium comprising variable numbers of fusiform initials and the intervening ray initials. The tangential extent of the cambium involved ranged from one to more than ten cells.

In *Thuja occidentalis*, *Juniperus communis* var. *depressa*, *J. horizontalis*, and *J. virginiana* the roots were conterminous to unusual xylem rays which differed from the ordinary rays in their larger size and, especially in *Thuja*, in the higher proportion of tracheary cells. The root meristems apparently origi-

nated in the phloem or cambial portions of these rays.

The roots in *Abies balsamea*, *Picea glauca* and *P. mariana* arose from dormant buds, but the distribution of the buds differed in the two genera. In *Abies* the roots were distributed irregularly along the layered branches, in *Picea* they were usually restricted to the neighborhood of the terminal bud scars.

In *Larix laricina* the adventitious roots originated in the vicinity of dead or injured dwarf branches. Layering was not discovered in *Tsuga canadensis* or in *Pinus* spp.

Examination of a few hormone-treated cuttings (*Picea*) indicated a different type of root origin from that described above for layered specimens.

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THE EXTRACTION OF AUXIN FROM PLANT TISSUES. II¹

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IT WAS shown in an earlier communication (Thimann and Skoog, 1940) that the bulk of the auxin in *Lemna* is present in a bound form, from which it is liberated slowly by long standing with ether. The extreme slowness of the extraction is not due to low solubility of auxin in the solvent, but is conditioned by the conversion of the auxin from an inactive to a free form. The evidence for this is: (1) the rate of liberation is independent of the volume of solvent used, (2) extraction is not hastened by maceration, (3) extraction is not hastened, but the liberation is actually prevented, by boiling, and (4) the presence of water is essential for the extraction process. In boiled material the bulk of the activity is lost and cannot be recovered however much the extraction period is prolonged; in dried material, on the other

hand, the subsequent addition of water allows recovery of a considerable part of the activity (in the case of *Avena coleoptiles*, all of the activity). It was deduced that the auxin is in some way combined with a protein, or a protein-like material, from which it is set free by hydrolysis. Support for this deduction was obtained by experiments, briefly reported, in which the extraction was greatly hastened by proteolytic enzymes (Skoog and Thimann, 1940).

Some trials with representative growing tissues indicated that the existence of such an auxin complex is of very general occurrence. As a matter of fact, evidence that auxin may exist in inactive bound form was given some time ago by Kögl, Erxleben, and Haagen-Smit (1934) when they showed that activity could be obtained from salad oil by treatment with lipase or by hydrolysis with acid. Re-

¹ Received for publication May 6, 1942.

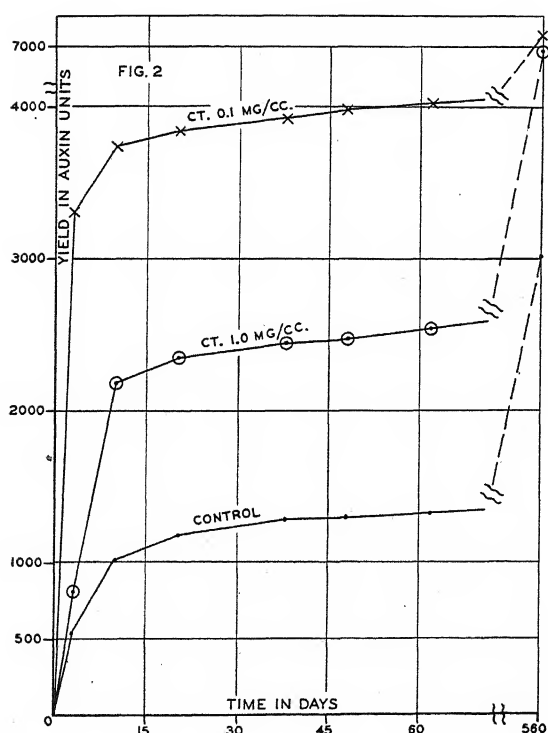
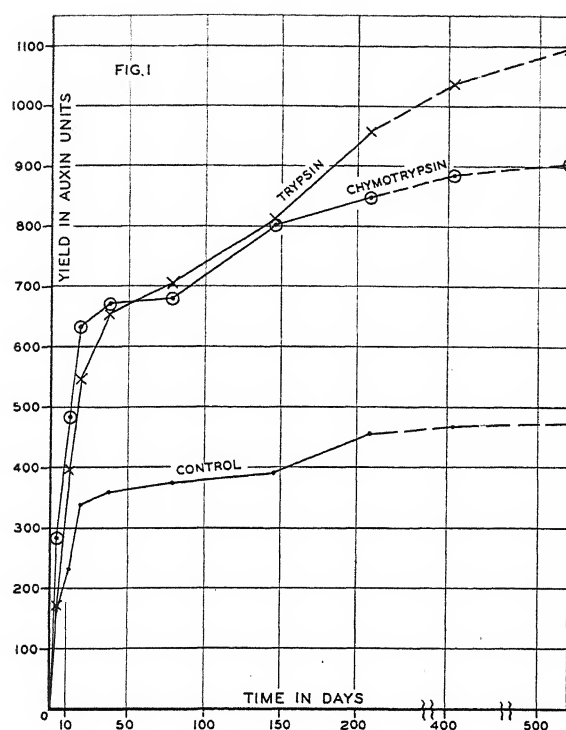


Fig. 1-2.—Fig. 1 (above). Dried *Lemna* powder (0.3 g. samples) incubated with and without 3 mg. of enzyme at 37° and pH 8.5 for twenty-four hours, then brought to pH 4 and given successive ether extractions in icebox. Begun May 4, 1940.—Fig. 2 (below). Two different con-

cently also Avery, Berger, and Shalucha (1941) have shown that the auxin in corn endosperm is set free by alkaline hydrolysis. It would appear, however, that the state of auxin in corn endosperm is entirely different from that in green tissues, investigated in our earlier experiments. Clear-cut evidence for this will be given below.

The present paper presents further evidence as to the nature of the auxin complex and brings out certain important considerations which will have to be taken into account before a reliable and convenient method for the quantitative extraction of auxin from plant tissues can be achieved. The standard *Avena* test was used throughout, and other procedures were as described earlier (Thimann and Skoog, 1940). All yields are expressed in the same arbitrary units as were used in the preceding paper, which are equivalent to fifty plant units.

EXPERIMENTS WITH ENZYMES.—Because of the indications, summarized above, that the material to which the auxin is bound may be of protein nature, experiments with proteolytic enzymes were undertaken.

Lemna, grown as previously described, was dried in a rapid current of warm air and ground to a fine powder. Samples of this powder were then wetted and incubated with varying amounts of proteolytic enzymes, the mixture being adjusted to the optimum pH of the enzyme with HCl and Na₂CO₃. Several temperatures between 25° and 50°C. were used, the periods of incubation being from one to five days. The solutions were afterwards acidified to about pH 4 (colorimetric) and then kept under ether, freshly distilled from FeSO₄, in the icebox. At intervals over a period of many months the ether was removed and renewed and the activity in the ether extracts determined as previously described. In preliminary experiments, crystalline trypsin and chymotrypsin (Kunitz and Northrop, 1935),² especially the latter, proved to be effective. Ficin (Walti, 1938) was less so, while papain, both with and without activation by HCN, was apparently inactive. Chymotrypsin was, therefore, selected for more extensive study.

The results of four comparable experiments are presented herewith. Two are given as figures 1 and 2, and two others as tables 1 and 2. Each point on the curves, or each figure in the tables, represents the best value for that particular extract as deduced from a number of *Avena* tests done at different dilutions. The total maximal yield varies with the particular sample of *Lemna* used, the extreme limits being usually 1,000 and 3,000 units per gram fresh weight (cf. Thimann and Skoog, 1940, p. 952), though the experiment of figure 1 is considerably

² The chymotrypsin preparation, obtained originally from Dr. Kunitz, was kindly supplied by Dr. A. A. Abramowitz.

concentrations of chymotrypsin (CT). Dried *Lemna* powder, 0.3 g. samples, incubated twenty-four hours at 37° and pH about 8.5, then brought to pH 4 and extracted with ether. Begun May 21, 1940.

TABLE 1. *Effect of chymotrypsin on extraction of auxin from Lemna. Dried powder, 0.3 g. samples (equal to 3 g. fresh weight), incubated with enzyme solution at pH 8.5 and 37° for twenty-four hours, then acidified. Extractions in icebox.*

Date of extraction	Days in each extraction or group of extractions	Control	Chymotrypsin, 1 mg.	Chymotrypsin, 5 mg.
May 16, 1940	2	500	2,288	1,275
May 23	7	170	680	613
Five extractions—May 31 to September 26..	124	2,819	3,066	3,829
Five extractions—October 17, 1940 to April 4, 1941	190	435	2,711	3,230
May 15, 1941	41	22	187	337
October 6	144	40	225	270
Total	508	3,986	9,157	9,554

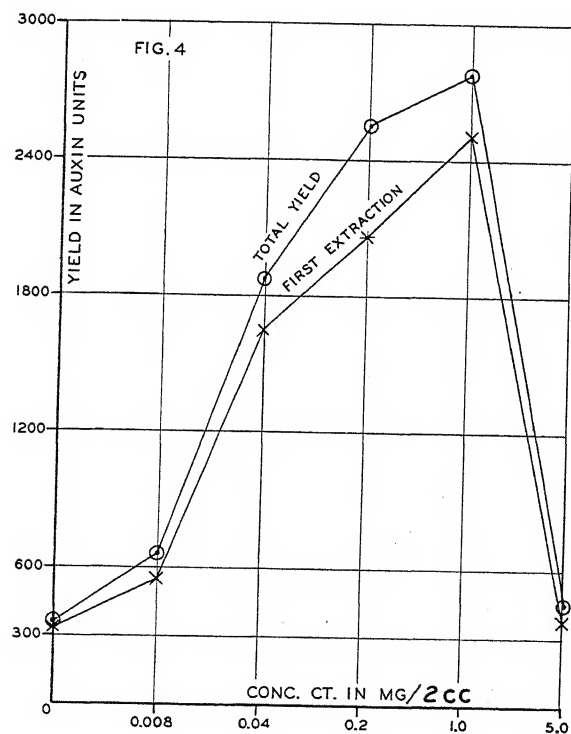
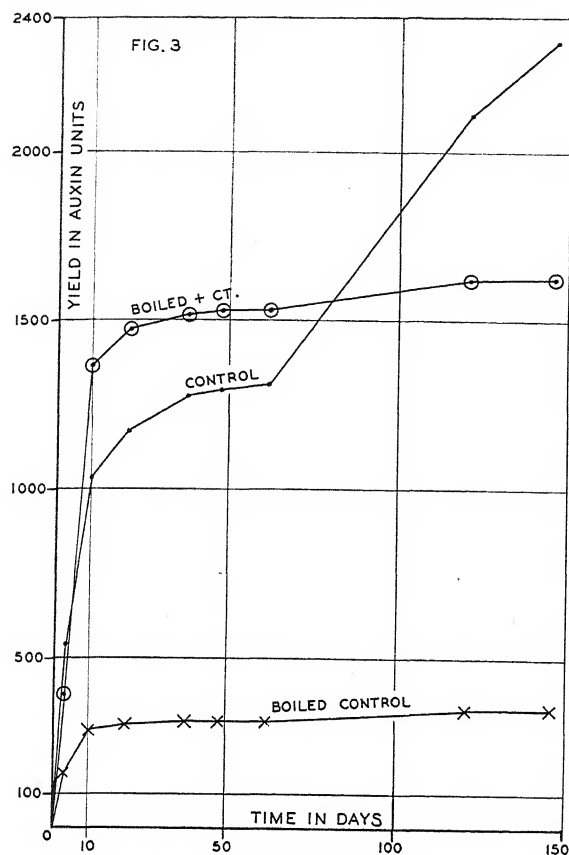
lower. The results show firstly, that in all experiments the yield of active auxin is greatly increased by the enzymes used. Secondly, in three of the four experiments shown, this increase takes place mainly in the earlier extractions. In later extractions the rates of liberation are slow and the controls may in some cases equal the rates in the enzyme-treated samples (see the last six extractions of table 2), while in other cases (table 1) the enzyme-treated samples show higher rates of liberation over the whole period. The slowness of liberation in later extractions is of course partly due to the fact that pH 6 and icebox temperature are far from optimal conditions for chymotrypsin. Thirdly, the time at which the rate of liberation decreases markedly is nearly the same with or without enzyme. This may be seen in figures 1 and 2 and also in table 2, in which after October 18 the activity of the enzyme seems exhausted. This result is surprising, since the added enzyme might be expected to set free rapidly what the control material could have set free slowly. This could be explained in two ways: (a) the added enzyme acts upon some additional source of auxin which was not liberated at all in the controls, (b) the bound auxin complex deteriorates with time in some way so that liberation becomes increasingly

difficult. The latter view is somewhat supported by the fact that dried *Lemna* powder on prolonged keeping loses a large part of its total extractable auxin. The general similarity in the shapes of the extraction curves with and without added enzyme is strong support for the view that the liberation of auxin in the controls is itself enzymatic.

The data previously reported (tables 4, 5 and 6 of Thimann and Skoog, 1940) show clearly that boiling prevents the subsequent slow liberation of auxin from *Lemna*. This effect of boiling may be merely the destruction of the enzyme, or it may be destruction of the auxin complex itself. If it is the former, it might be possible to liberate the auxin from boiled material by adding proteolytic enzymes. Accordingly, samples of the powder were boiled for five minutes in water and then treated with chymotrypsin as before. The results of one such experiment are shown in figure 3. This experiment was carried out at the same time as the experiment of figure 2. At the end of the third extraction, after which practically all the auxin had been removed from the boiled material, the unboiled control had yielded a total of 1,176 units, while the boiled sample treated with chymotrypsin had given 1,455. Thus chymotrypsin added to the boiled material has raised the

TABLE 2. *Experiment similar to that of table 1.*

Date of extraction	Days in each extraction	Control (mean of 2)	Chymotrypsin, 0.1 mg.	Chymotrypsin, 1 mg.
June 25, 1940	1	280	925	413
July 8	13	79	263	94
July 22	14	59	245	66
September 26	65	632	950	2,080
October 18	22	98	143	550
November 7	20	50	67	60
December 23	46	31	22	30
February 10, 1941	49	22	58	56
April 4	53	62	75	74
May 15	41	60	60	37
October 9	147	124	135	120
December 1	53	51	105	39
Total	528 days	1,548 units	3,048 units	3,619 units



yield to a value even higher than that of the unboiled control. It will be seen from the curve that later extractions continued to set free appreciable amounts (some 200 units) in presence of chymotrypsin but practically none in the plain boiled control. (The unboiled control in the same period, however, yielded about 1,000 additional units.) Thus the enzyme added at the start of the experiment continues to exert a definite though small effect over a long period of time. It may be concluded that, at least in part, the added proteolytic enzyme can replace the agent which is inactivated by boiling. However, since the total yield is only about one-fourth³ that set free by chymotrypsin from unboiled material in the same experiment, the second possibility, *i.e.*, that the auxin complex has been irreversibly changed, is by no means excluded. It is of interest to note that according to Gustafson (1941), the free auxin is not inactivated by brief boiling.

INFLUENCE OF ENZYME CONCENTRATION.—In an attempt to obtain optimum yields, the influence of the concentration of enzymes was studied. It will be noticed in figure 2 that of the two concentrations of chymotrypsin used, the lower showed the greater activity, at least at first. The same is seen in tables 1 and 2, but here the higher concentration gradually recovers the lead to reach about the same final value. This effect was found consistently and indicates that for some reason there is an optimum concentration of chymotrypsin. A more careful study of this factor is shown in figure 4. In this experiment the enzyme preparation, which is rich in $MgSO_4$, was dialyzed against distilled water before use, but this treatment seems to have had very little effect. The results show excellent proportionality between auxin liberated and enzyme concentration up to a well-marked optimum at 0.5 mg. per cc. The reason for this optimum has not been investigated, but incidentally it affords evidence that the enzyme preparation does not itself supply any auxin. This confirms negative auxin tests on three samples of enzyme, both freshly prepared and after incubation as above.

Results of a similar experiment with crystalline ficin (Merck) are given in table 3. Again there is a good indication of proportionality with enzyme concentration, and a clear-cut optimum. The ficin alone contained no auxin.

THE INFLUENCE OF TREATMENT FAVORING ACTION OF AUTOGENOUS ENZYMES.—In view of the above results with added enzyme, attempts were next undertaken to hasten the auxin-liberating activity of en-

³ In another experiment the ratio was one-third.

Fig. 3-4.—Fig. 3 (above). As figure 2, but *Lemna* powder boiled ten minutes in water before addition of enzyme. (The rise in extracted auxin in the unboiled control after sixty-two days is unexplained.) Begun May 21, 1940.—Fig. 4 (below). Effect of different concentrations of chymotrypsin (CT), dialyzed for twenty hours before use. Dried *Lemna* powder, 0.1 g. samples, incubated forty-eight hours at 37° and pH 8.5, then brought to pH 4 and extracted with ether. Three days for first extraction. Total yield based on subsequent extractions over six months in icebox. Begun October 5, 1940.

TABLE 3. *Liberation of auxin by crystalline ficin. Dried Lemna powder, 0.1 g. samples (=1.0 g. fresh weight), incubated forty-eight hours at 29° and pH 5 with the enzyme, then extracted with ether in icebox for three days, and a further four days.*

Weight of ficin (mg.)	Auxin units (sum of two extractions over 7 days)
0	389
0.1	613
1	1,255
10	105
5 (no <i>Lemna</i>)	0

zymes presumably present in the plant tissue itself. This was done by prolonged incubation of the moist plant material at a favorable temperature. In the first experiments of this type, enormous but very variable yields were obtained, values as high as 40,000 units per gram of *Lemna* (as compared with the normal figure of 1,000 to 3,000) being reached. Associated with this was the presence of a large population of bacteria. Frequently, the high yields were correlated with the total bacterial population as determined by counts on nutrient agar plates. On the other hand, an occasional high bacterial count was associated with a very low yield, so that it is evident that bacterial contamination can both produce and destroy auxin. Clearly, successful incubation requires aseptic or at least antiseptic conditions. Using plate counts in parallel with auxin determinations, a large number of antiseptics was studied, but in the few cases where these were completely effective in stopping bacterial growth, the presence of the antiseptic interfered seriously with the subsequent auxin tests.

A few experiments with *Lemna* which had been grown in bacteria-free culture, prepared and incubated under aseptic conditions, and checked for sterility afterwards by bacterial counts, gave inconclusive results and will not be reported in detail. Since this method would in any case not be of general application for the determination of auxin, it seemed preferable to concentrate attention upon the use of antiseptics. Of these, phenol was the only one which completely suppressed bacterial growth and

at the same time gave consistent figures for auxin content. A serious disadvantage of the use of phenol is that it causes extensive destruction of the auxin. This is true even when pure phenol is incubated with pure indole-acetic acid and the solution afterwards extracted with ether, as is shown by table 4. Never-

TABLE 4. *Destructive effect of incubating pure indole-acetic acid with phenol. All vessels brought to pH 3 before extraction. Each figure the mean of two samples.*

Time incubated	Concentration of phenol	Units extracted (sum of 2 extractions over 6 days)
Three days in icebox.....	0	22,750
Three days in icebox.....	2%	22,500
Three days at 50°.....	0	20,900
Three days at 50°.....	2%	1,200

theless, although the yields obtained are much less than the true total auxin, this method does at least allow a demonstration of the effects of incubation and of added enzyme. In the two experiments of table 5 fresh *Lemna* was suspended in 2 per cent phenol. The figures show that auxin is steadily liberated with increasing time of incubation. As might be expected, 30° is more effective than 50°.

As a matter of fact, very similar results both qualitatively and quantitatively were obtained in several experiments in which appreciable bacterial contamination did not occur. In the two experiments

TABLE 6. *The release of auxin by short incubation of Lemna without phenol. Results of a single extraction two days after treatment. Duplicate samples, 0.1 g. dry powder (= 1 g. fresh weight); no phenol.*

Treatment	Units
Icebox	35
One day at 30°.....	250
One day at 60°.....	165
Same + chymotrypsin 0.1 mg. (pH adjusted as in table 1)	
Icebox	153
One day at 30°.....	435
One day at 50°.....	159

TABLE 5. *Showing that incubation even in presence of phenol increases the amount of auxin subsequently extractable. Samples, 1 g. fresh Lemna in each case, all with phenol 2 per cent, lightly acidified before extraction. Each figure the mean of a pair of duplicates.*

Temperature	Incubation time (days)	Sum of 3 extractions (3½ months)	Two further extractions (3 months)	Two further extractions (4 months)	Total	Another experiment; 4 extractions over 5 months, total
Icebox	1	53	17	0	70	47
50°	1	85	36	2	123	100
50°	3	134	1	0	135	...
50°	5	180	0	0	180	149
30°	3	194	41	5	240	...
30°	5	241	30	4	275	...

TABLE 7. Tobacco leaves. Influence of incubation with enzyme, in presence of phenol; pH adjustment as in table 1. Figures for icebox and two-day incubation are the mean of duplicates. Compare with table 6.

Temperature	Incubation Time	Phenol	Chymotrypsin added, mg.	Auxin in first extraction	Total auxin in 7 extractions over 9 months
Icebox	2 days	0	0	4	79
40°	2 days	2%	0	10	14
40°	2 days	2%	0.1	64	227
40°	5 days	2%	0	5	10
40°	5 days	2%	0.1	118	239

summarized in table 6, phenol was not added. It is clear that both with and without added chymotrypsin, the yield of auxin is increased considerably by incubating at 30°, and to a less extent at 50°. In experiments with phenol, auxin does not continue to appear after the first two or three extractions (see table 5), although the phenol has been entirely removed in the first ether extraction. This may indicate a destructive effect on the auxin reserves in the tissue.

The experiments are in the main thoroughly consistent with the view that the normal liberation of auxin from *Lemna* kept under ether is enzymatic, but unfortunately they do not prove it conclusively. There is apparently a purely physical effect of incubation on the liberation of auxin. In one experiment a slight but definite increase was obtained on incubating sterile and autoclaved *Lemna*. A clear-cut decision on this point has not been reached and much more extensive experimentation would be required to do so.

Results similar to those with *Lemna* were obtained on leaves, using *Nicotiana tabacum*, grown in the greenhouse (table 7). Fresh leaves, cut into medium sized pieces, were incubated two to five days with and without enzyme. The experiment shows again

that, although phenol causes some destruction, incubation with chymotrypsin even in the presence of phenol greatly increases the yield of free auxin.

GENERAL CONSIDERATIONS OF THE SERIAL EXTRACTION METHOD.—In all our experiments it has been clear that complete extraction can only be obtained by very numerous serial extractions spread over a long period of time. In every extraction there is a risk of some loss; it was, therefore, important to determine whether the extraction procedure in itself destroys any auxin. The following data show that it does.

In the first place, there is a general tendency for the yield per gram of *Lemna* to be greater with large than with small samples. This in itself is suggestive but not conclusive. In the second place, however, direct experiments in which two duplicate samples were extracted, one frequently and one infrequently, show that the total yield is considerably greater in the latter. One such experiment is given in table 8.

A similar loss by frequent extraction was shown in an experiment carried out with the alga, *Ulva lactuca*, freshly collected from the shore. Auxin contents of some marine algae have been reported by van der Weij (1933) and van Overbeek (1940),

TABLE 8. Influence of frequency of extraction. Fresh *Lemna*, 3 g. samples. Placed in ether November 30, 1940.

Date of extraction	No. 1		No. 2	
	Days in each extraction	Units in each extraction	Days in each extraction	Units in each extraction
December 1, 1940.....	1	44
December 5	4	115	5	105
December 9	4	205
December 12	3	62
December 16	4	106	11	365
December 26	10	102	10	575
January 10, 1941	15	66
January 20	10	26
January 31	11	21
March 20	48	15	84	860
June 6	78	9	78	63
January 12, 1942	6 months	0	6 months	200
Total	12.5 months, 12 extractions	771 units	12.5 months, 6 extractions	2,168 units

TABLE 9. Influence of frequency of extraction. *Ulva lactuca*. Matched samples, 5 g. fresh weight. Placed in ether October 4, 1941.

Date of extraction	No. 1			No. 2		
	Days in each extraction	Units in each extraction	Units per day	Days in each extraction	Units in each extraction	Units per day
October 11, 1940.....	7	17	2.4
October 14.....	3	13	4.3
October 18.....	4	9	2.2
October 28.....	10	66	6.6	24	205	8.5
November 8.....	11	27	2.5
November 29.....	21	43	2.0	32	146	4.6
December 9.....	10	16	1.6
January 10, 1941.....	32	34	1.1
January 17.....	7	20	2.8
January 24.....	7	9	1.3	56	78	1.4
February 14.....	21	13	0.6
March 27.....	41	9	0.2	62	10	0.2
June 12.....	77	2	0.03	77	5	0.07
November 9.....	119	0	0	119	0	0
Total.....	370 days, 14 extractions	278 units		370 days, 6 extractions	444 units	

using a single extraction or a short-time Soxhlet extraction with ether, and by duBuy and Olson (1937) using a single extraction with chloroform. Our figures are given here (table 9), because they show that also in this material the general process of auxin liberation closely resembles that in the leaves of higher plants, and determination of total auxin can only be made by prolonged extraction. It will be seen from the first series that a period of about eight

weeks in the icebox is required to obtain 75 per cent of the total yield. The second series shows that, as with *Lemna*, a few extractions, widely spaced, give higher yields. This is mainly true in the first eight weeks while the amount of auxin is still considerable. It must, unfortunately, be concluded that each extraction causes appreciable loss of total activity.

These considerations suggest that a first approximation to the determination of total auxin can be

TABLE 10. Tomato leaves. Matched samples. Dry material extracted with ether thoroughly dried over sodium in all cases; fresh material extracted with moist ether. See text.

Dates of extraction:		4/7/41	4/11	6/9		10/23	12/17	2/2/42		
Material		Auxin units extracted								
A										
Leaflets, wgt. 5.7 g.	Fresh	135	250	410	122	20	10		
	Dried slowly	364	675	1,852	Wetted	3,930	390	49		
Petioles, wgt. 5.0 g.	Fresh	20	...	24	0	2	0		
	Dried slowly	15	...	184	Wetted	219	9	1		
Dates of extraction:		4/14/41	4/18	4/28		5/22	5/29	6/23	9/29	12/11
B										
Leaflets, wgt. 1.6 g.	Fresh	194	165	163	12	2	120	4	0
	Frozen, then dried quickly	2	3	14	Wetted	44	22	55	14	0
	Boiled	0	0	0	1	10	27	0
Dates of extraction:		9/29/41	10/16			3 extractions to 1/2/42		Total		
C										
Leaflets, wgt. 3.0 g.	Fresh	1,313	62		15	39	1,429		
	Frozen, then dried quickly (mean of four)	17	115	{ 2 wetted 2 left dry		47 0	95 4	274 136		
	Autoclaved	13	8		2	6	29		
	Dried slowly	88	4	Wetted		9	38	139		

had by covering the fresh material with peroxide-free ether, leaving it in the icebox for about eight weeks, and determining the auxin in the ether extract and washings. After covering the material with fresh ether, a second extraction should be made a few weeks later. The sum of these two extractions will in most instances add up to at least 75 per cent of the total extractable auxin. Reference to table 11 of the previous publication (1940) will show that this procedure will probably suffice for roots, while the controls of figures 2 and 3 above show the same thing for *Lemna*, and the controls of table 10 for leaves.

Another important influence is that of water. It was shown in Part I (1940) that, in *Avena* coleoptiles and roots as well as in *Lemna*, thorough drying prevents the extraction of auxin by ether. On adding water, auxin may again be extracted. In these experiments, the ether extract from the dry material was freed from ether and taken up in aqueous agar in the usual way. Subsequently, however, it was stated by Link (1940) that this ether may contain a precursor from which auxin is set free by simple warming with water. This was not confirmed (Link, Eggers, and Moulton, 1941), and the experiment below shows also that it is not the case; water sets free nothing from the ether extract. From the dried *Lemna* itself, however, water sets free extractable auxin in the usual way. This confirms (if further confirmation be needed) the necessity of water for the extraction process.

The experiment was as follows: Fresh *Lemna* (15 g.) was thoroughly washed, dried first in a stream of hot air and then overnight in the oven at 50°, and then extracted for eight days with thoroughly dry ether (freshly distilled over sodium). The ether extract was divided into two equal parts, both evaporated to dryness, and the residue of one part (b) gently warmed with water. Subsequently both (a) and (b) were taken up in agar and tested. To 0.5 g. of the residual dry *Lemna* (corresponding to 5 g. fresh material) 0.5 cc. water was added and the moist product again extracted with ether (c).

(a) activity of dry ether extract of dry *Lemna*.... 0 units
(b) same, after reacting with water..... 0 units
(c) ether extract of moistened *Lemna*..... 50 units

Although all our experiments on *Lemna* and *Avena* agree in showing the necessity of water for auxin extraction, experiments with leaves are not always consistent. Thus Went (private communication), in repeating our experiments with tomato leaves, actually obtained more auxin from dried than from fresh material. Section A of table 10 shows a similar result. Though wetting appears to release some more auxin, the dried material yields more than the fresh. In these experiments the leaves were dried in a current of warm air for thirty-six hours, followed by an overnight stay in the oven at 40°. If the liberation of auxin is enzymatic, such a protracted exposure to warmth while still moist would allow extensive liberation. It would also allow the development of microorganisms.

After numerous attempts to hasten the drying, it was found that if the leaves were first frozen in solid CO₂ they could subsequently be dried in a few hours instead of requiring two days (cf. also Link, Eggers and Moulton, 1941). Section B of table 10 shows that such leaves, frozen and then quickly dried, behave in the manner found in all our earlier experiments. Only traces of auxin are extracted while the material remains dry. On adding water the yield increases, but does not reach that of the fresh material. Drying, in other words, causes a considerable permanent inactivation of the auxin. In such experiments it must be noted that dried ether will undoubtedly absorb water in the icebox, even in a closed vessel. Section C of table 10 presents a similar experiment; although the data from material dried slowly are not clear, the contrast between fresh and rapidly dried leaves is perfectly definite, and the subsequent addition of water has the expected effect. The figures for heated material are included for completeness; they serve only to confirm for leaves the previous findings on other material that heating almost completely prevents the extraction of auxin.

In general it can be concluded that leaves do not differ in principle from the other material studied, though they are more difficult to dry, and perhaps more sensitive to small amounts of water.

COMPARISON WITH CORN ENDOSPERM.—The ready liberation of auxin from corn endosperm by alkaline autoclaving (Avery, Berger, and Shalucha, 1941) contrasts sharply with the behavior of *Lemna*, in which all our experiments have shown that heating to 100° at any pH produces only destruction of the auxin. To make this point clear, a comparative experiment was carried out in which the endosperm and *Lemna* were given the same treatments side by side.

A sample of dry field corn (Yellow Dent), freed from the embryos, was ground in a ball mill and three pairs of duplicate 0.5 gram samples treated as follows:

- (1) Extracted for three days with 5 cc. H₂O.
- (2) Brought to pH 9 with dilute NaOH, autoclaved thirty minutes at 120°.
- (3) Extracted for three days with moist ether in presence of a few drops of dilute acetic acid.

In the first two pairs, the water extract, after filtering, washing and bringing to pH 4, was tested directly; in the third the ether was evaporated off

TABLE 11. Comparison of *Lemna* powder with corn endosperm. Each figure is the mean of two 0.5 g. samples. For details see text.

Treatment	Auxin units extracted	
	Corn endosperm	<i>Lemna</i> powder
Extracted with water	380	0
Autoclaved at pH 9, then cooled and acidified	7,080	0
Moistened and extracted with ether	291	92

and the residue taken up in 0.5 cc. warm agar in the usual way. Dried *Lemna* powder (paired 0.5 g. samples) was treated in the same three ways. The results are given in table 11. It is clear that in the corn, water treatment extracts even more auxin than ether (as was shown by Avery, Creighton and Shalucha, 1940), and alkaline autoclaving increases the yield some 2,000 per cent. In *Lemna*, water does not extract any detectable auxin and alkaline autoclaving has no effect, while ether will extract a considerable amount. The contrast is complete. It may be noticed also that, although no attempt was made to obtain the maximum yield, the absolute amount of auxin obtained in the autoclaved corn is comparable with that found by Avery, Berger and Shalucha, for our units are equivalent to fifty plant units, and since one-half gram samples are used, our figure should be multiplied by one hundred to give total plant units, and by a further factor of four to give "total degrees curvature" of these authors' notation. The value is thus some three-quarters of a million plant units, or three million "total degrees curvature," per gram.

SUMMARY

The yield of auxin from *Lemna* is increased greatly by short incubation with chymotrypsin or trypsin. Ficin has a similar, though smaller effect.

The same is true for leaves of tobacco.

Although the yield is very greatly decreased by boiling, treatment with proteolytic enzymes raises the yield from boiled material to a value comparable with that obtainable from the unboiled.

It is concluded that the auxin in green material is bound to protein or a protein-like substance, and that the effect of boiling, while it may to some extent destroy the complex itself, is in the main to destroy the enzyme system which liberates the auxin.

On the other hand, prolonged incubation of plant material to allow the autogenous enzymes to liberate the auxin is not a practicable general procedure for determination of total auxin, on account of the large and variable production of auxin by bacteria. Addition of bactericides cannot completely overcome this difficulty.

Incubation of plant material, or of pure indoleacetic acid, with phenol leads to extensive destruction of the auxin activity. In spite of this, some auxin-liberating action of enzyme occurs even in phenol.

Ulva lactuca, a green alga, behaves like the leaves of higher plants in that its auxin is liberated to ether only with extreme slowness.

Tomato leaves are shown to behave like the materials previously studied in that water is necessary to allow the extraction of auxin, provided only that the leaves are dried rapidly. During slow drying, auxin may in some cases be produced, either by bacteria or by enzymatic liberation.

In serial extractions with ether, each extraction destroys a part of the auxin which has been liberated.

Unlike corn endosperm, no auxin is liberated from *Lemna* either by extraction with water or by alkaline autoclaving. The bound forms of auxin in these two plant materials are, therefore, of quite different types.

Suggestions for a simplified procedure are put forward which will determine the bulk of the total auxin in green plant material with a minimum number of extractions.

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WHEN BOILED grass leaves are used as bait for the saprophytic chytrids in soil and water collections, *Pythium* is often found growing on the grass leaves. This method of collecting has proved effective in obtaining a number of species of *Pythium* and parasites of *Pythium*, which frequently are species of *Pseudolpidium* or *Olpidiopsis*. *Pseudolpidium gracile* Butler, the resting bodies of which lack companion cells, has been recollected and studied. In addition, two new species of *Olpidiopsis*, the resting bodies of which possess companion cells, have been found parasitizing *Pythium*. In a number of collections, however, the grass leaf bait yielded a species of *Aphanomyces* which was parasitized by what appeared to be *Pseudolpidium Aphanomyces*, in that all the resting bodies were without companion cells. These four species of *Pseudolpidium* and *Olpidiopsis* will be discussed in this paper.

Recently taxonomic studies of the genus *Olpidiopsis* have been published by Shanor (1939, 1940) and McLarty (1939, 1941). Shanor (1939) suggests that the genus *Pseudolpidium* should be combined with *Olpidiopsis* for "none of the species originally placed in the genus when it was established can remain unquestionably as *Pseudolpidium* species. The continuance of the genus seems to rest on only two species, *P. Pythii* and *P. gracile*, both described by Butler. Even these two species need further critical study." McLarty (1941) merges the genus *Pseudolpidium* with the genus *Olpidiopsis* and amends *Olpidiopsis* "to include species with smooth and spiny zoosporangia which may form either sexual or asexual spores." Of *Pseudolpidium Pythii* and *P. gracile* McLarty states, "Very little is known of these species, although each has been observed since Butler's time. The resting spores of these species, as they are illustrated in the literature, appear very similar in appearance to spiny zoosporangia. Until they are re-collected and critically examined, these species may be transferred to *Olpidiopsis* as strictly asexual species."

No opinion is expressed here as to the validity of the genus *Pseudolpidium*. The combining of *Pseudolpidium* and *Olpidiopsis* by McLarty (1941) on the basis of his having found both sexual and asexual spores in monospore cultures of his species, *Olpidiopsis Achlyae*, is justifiable. Nevertheless, the studies presented in this paper will emphasize the fact that in certain forms, formerly considered as species of *Pseudolpidium* and now to be placed in *Olpidiopsis*, according to McLarty, the lack of a companion cell on the resting spore is a constant and consistent characteristic of the species. In this connection two species, *Pseudolpidium gracile* Butler and *P. Aphanomyces* (Cornu) Fischer are now to be discussed.

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Pseudolpidium gracile Butler.—A soil collection made by Dr. H. R. Totten at Key Largo, Florida, on December 27, 1940, yielded *Pythium rostratum* (fig. 15-20), parasitized by a species of *Pseudolpidium* (fig. 7 and 8). Because of the length of the spines and the yellowish color of the resting bodies, the parasite was identified as *Pseudolpidium gracile* Butler, though Butler (1907) lists *Pythium rostratum* as the host of *Pseudolpidium Pythii*. *Pythium intermedium* is given as the host of both *P. Pythii* and *P. gracile*, so it is possible that *Pythium rostratum* is likewise susceptible to both species of *Pseudolpidium*. *Pseudolpidium gracile* has been kept under observation in culture on its host since the date of its collection. Resting bodies are constantly produced in large numbers, but not a single resting body with a companion cell has been seen. The resting body is yellowish in color and covered with slender spines which attain a maximal length of 4 μ (fig. 3). In shape the resting body varies from spherical to oval and measures 12 to 27 μ in diameter, exclusive of the spines. Butler states that the wall of the resting body of his species is rather thin and in his figures of the resting body the wall does appear to be very thin. It is for this reason that Shanor and McLarty have suggested that what Butler figures as resting bodies may be only spiny-walled zoosporangia. It is possible that Butler drew the resting bodies before the wall had attained its mature thickness for the contents of the resting body as illustrated by Butler appear to be undifferentiated. A mature resting body from the cultures of our *P. gracile* contains a single central oil globule surrounded by a peripheral layer of vacuolated protoplasm (fig. 3). Butler does not give any measurements of the thickness of the exospore and endospore walls of the resting bodies of *P. gracile* but measurements made on the resting bodies in our cultures show that the endospore may attain a thickness of 0.7 to 1.2 μ while the exospore varies from 1.7 to 2.5 μ in thickness.

The development of the zoosporangia and the discharge of the spores do not differ from the descriptions of Butler. A period of vacuolization, during which one or two exit papillae appear on the sporangium, is terminated by the sudden disappearance of the vacuoles. Very soon after the disappearance stage the spores are cut out and discharged. The zoospores are biflagellate. The walls of some of the zoosporangia in older cultures are often covered with very short, fine spines (fig. 9).

Pseudolpidium Aphanomyces (Cornu) Fischer.—A culture of *Aphanomyces cladogamous* Dreschler was obtained from soil collected by Philip Couch near Petahatche, Mississippi. The *A. cladogamous* was parasitized by a species of *Olpidiopsis* which formed resting bodies that lacked companion cells (fig. 4 and 24). The identification of this species constitutes a difficult problem for two species of

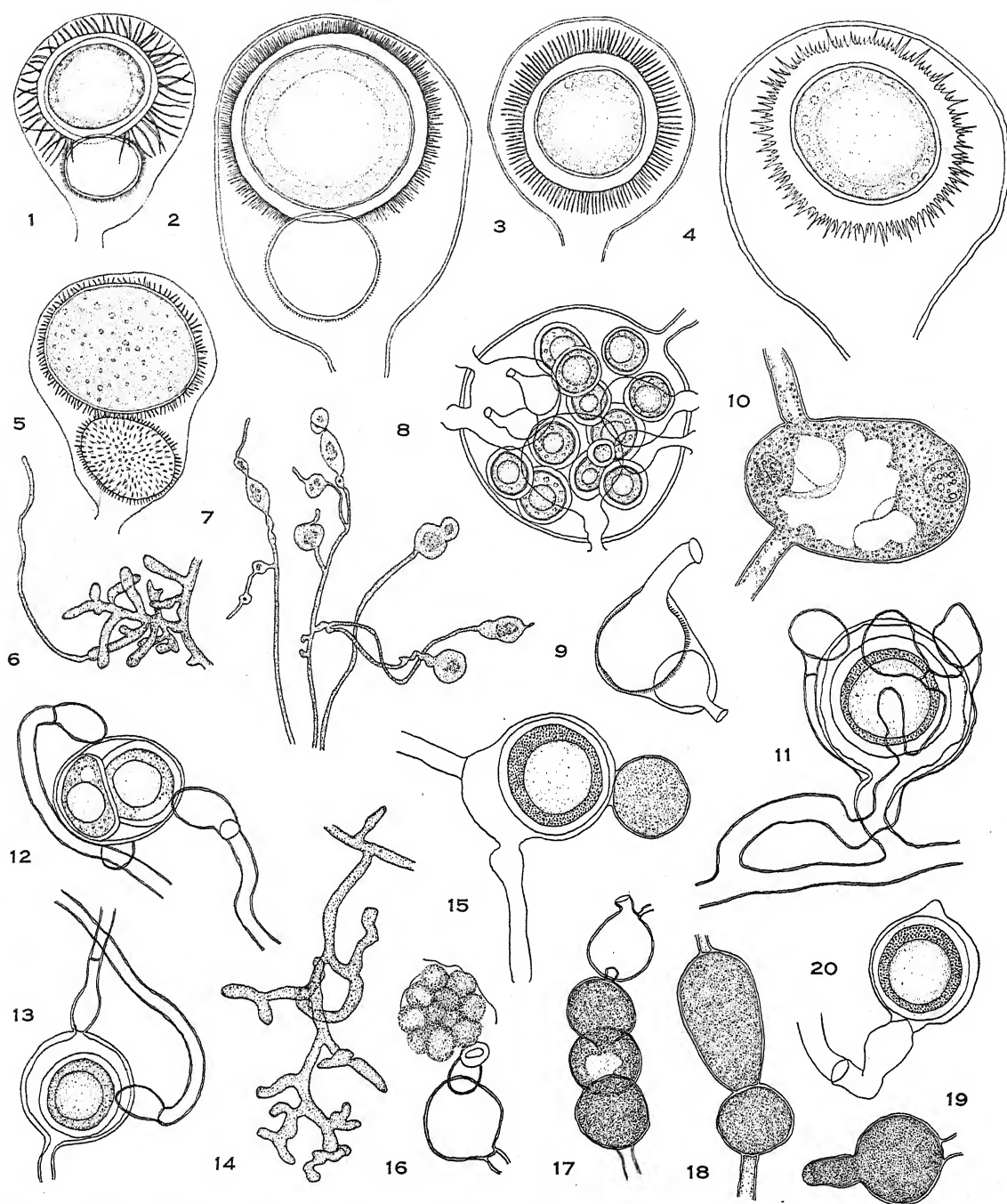


Fig. 1-20.—Fig. 1. Resting body of *O. curvispinosa*, $\times 850$.—Fig. 2. Resting body of *O. brevispinosa*, $\times 850$.—Fig. 3. Resting body of *P. gracile*, $\times 850$.—Fig. 4. Resting body of *O. Aphanomyces*, $\times 600$.—Fig. 5. Spiny-walled zoosporangia of *O. curvispinosa*, $\times 600$.—Fig. 6. Zoosporangium of host of *O. curvispinosa*, $\times 200$.—Fig. 7. *Pythium rostratum* parasitized by *P. gracile*, $\times 110$.—Fig. 8. Smooth-walled resting bodies of *P. gracile*, $\times 600$.—Fig. 9. Spiny-walled zoosporangium of *P. gracile*, $\times 300$.—Fig. 10. Unilateral swelling of the hypha of *A. cladogamous* caused by *O. Aphanomyces*, $\times 300$.—Fig. 11. Oogonium and antheridia of *Aphanomyces cladogamous*, $\times 850$.—Fig. 12 and 13. Sex organs of *Pythium* sp., host of *O. brevispinosa*, $\times 850$.—Fig. 14. Zoosporangium of host of *O. brevispinosa*, $\times 300$.—Fig. 15-20. *Pythium rostratum*.—Fig. 15 and 20. Sex organs, $\times 850$.—Fig. 16. Zoosporangium discharging spores, $\times 530$.—Fig. 17 and 18. Conidia and zoosporangia, $\times 530$.—Fig. 19. Conidium germinating, $\times 530$.

Pseudolpidium or *Olpidiopsis* have been reported as parasites of *Aphanomyces*. *Olpidiopsis luxurians*, described by Barrett (1912) and restudied by

Shanor (1939), is a well defined species, having companion cells on the resting bodies, which parasitizes *Aphanomyces laevis*. *Pseudolpidium Apha-*

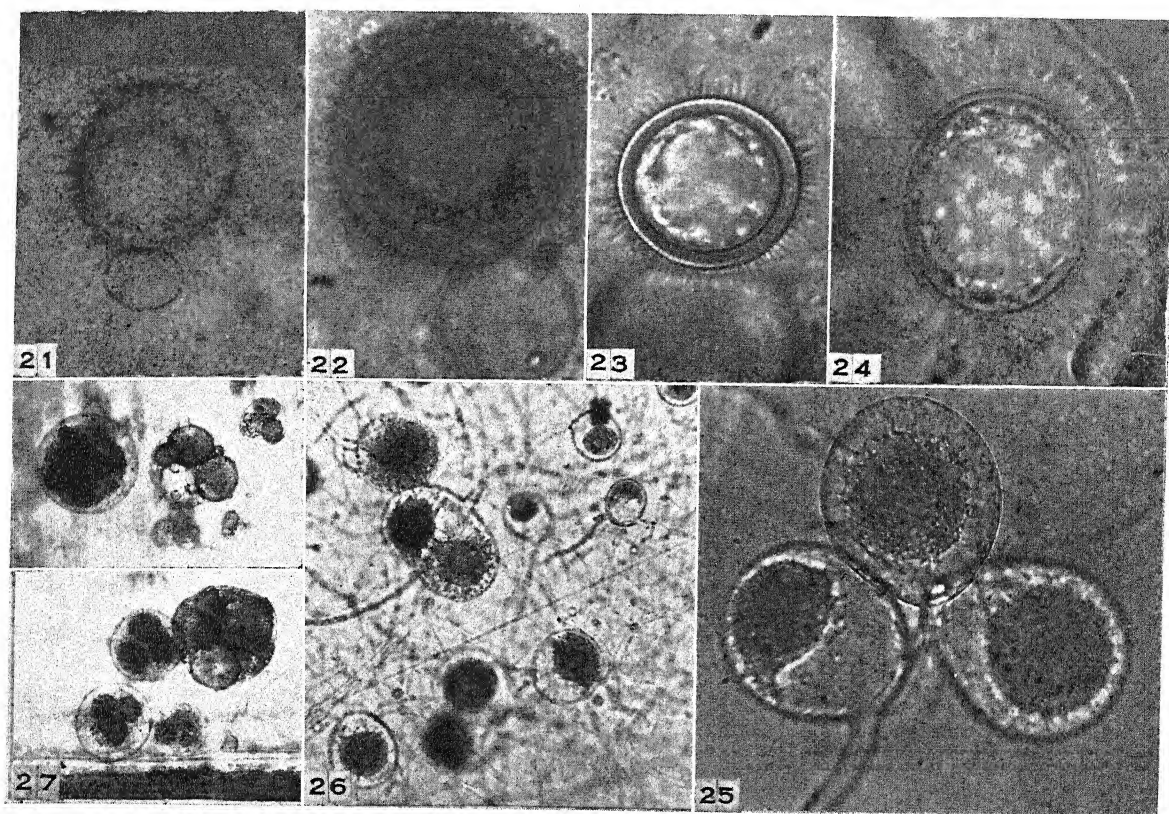


Fig. 21-27.—Fig. 21. Resting body of *O. curvispinosa*, $\times 430$.—Fig. 22. Resting body of *O. brevispinosa*, $\times 400$.—Fig. 23. Resting body of *P. gracile*, $\times 430$.—Fig. 24. Resting body of *O. Aphanomyces*, $\times 270$.—Fig. 25 and 26. *Olpidiopsis Aphanomyces* on *A. cladogamous*. Fig. 25, $\times 150$; fig. 26, $\times 100$.—Fig. 27. Swellings of *Pythium* sp. caused by *O. brevispinosa*, $\times 100$.

nomycis (Cornu) Fischer was described by Cornu (1872) as a species of *Olpidiopsis* even though he had found only the zoosporangia in an unidentified species of *Aphanomyces*. Fischer (1892) transferred this species to *Pseudolpidium* without having observed the resting bodies. Subsequently, Dangeard (1891) illustrated a resting body without a companion cell that he found parasitizing *Aphanomyces*, and Petersen (1910) described resting bodies with companion cells in the hyphae of a species of *Aphanomyces*. McLarty (1941) would combine the parasites of *Aphanomyces* observed by Cornu, Dangeard, and Petersen under Cornu's original name, *O. Aphanomyces*. It is not possible to know whether Cornu described as *O. Aphanomyces* the zoosporangia of *O. luxurians*, the present species, or some other form of *Olpidiopsis*. Yet it seems preferable now not to describe a new species of *Olpidiopsis* on *Aphanomyces* but to recognize the form on *A. cladogamous* as *Olpidiopsis Aphanomyces* Cornu.

OLPIDIOPSIS APHANOMYCIS Cornu

Pseudolpidium Aphanomyces (Cornu) Fischer

Zoosporangia 34.2 to $94.05\ \mu$, one or two, sometimes more in swellings of the host hyphae which are usually unilateral. Zoospores biflagellate. Resting

bodies oval, $24.6 \times 28.7\ \mu$ to $36.9 \times 43.05\ \mu$, or spherical, 13.2 to $44.46\ \mu$, covered with broad-based spines, 2.1 to $4.1\ \mu$ in length; exospore wall $4.1\ \mu$ thick, endospore wall $2.3\ \mu$ thick; companion cell lacking.

Parasitic in *Aphanomyces cladogamous* Dreschler.

Characteristic of *Olpidiopsis Aphanomyces* and *O. luxurians* (Shanor, 1939), as well, is the unilateral character of the swelling caused by these two parasites (fig. 10, 25, and 26). The resting bodies are formed in great abundance but require a greater length of time for maturation than those of *Pseudolpidium gracile*.

Olpidiopsis curvispinosa n. sp. and *Olpidiopsis brevispinosa* n. sp.—Up to the present time no other species of *Olpidiopsis* than *O. gracile* and *O. Pythii* have been described as occurring on *Pythium*. Isolation from soil collections was made of two species of *Pythium*, each of which was parasitized by a species of *Olpidiopsis*. The first species of *Olpidiopsis* forms resting bodies with long curved spines with a companion cell that may or may not be spiny (fig. 1 and 21) and is named *O. curvispinosa*. The second species has large, brownish resting bodies with fine short spines and a usually smooth companion cell (fig. 2 and 22) and is called *O. brevispinosa*.

OLPIDIOPSIS curvispinosa sp. nov.²

Zoosporangii globosis vel ovalibus, 12–68 μ diametro, pariete levi vel cum spinis brevibus. Zoosporis biflagellatis. Spora perdurante globosa vel ovali, 17–24 μ diametro, cellula adhaerente praedita. Episporio cum spinis curvatus usque ad 5 μ longis. Cellula adhaerente 14–20 μ diametro, levi vel cum spinis brevibus.

OLPIDIOPSIS curvispinosa n. sp.

Zoosporangia one to many in terminal or intercalary swellings of the host hyphae, spherical to oval in shape, 12 to 68 μ in longest diameter, cellulose wall smooth or with short bristles, one to three exit tubes. Zoospores with numerous small oil globules, elongate and somewhat reniform, biflagellate, flagellae of about equal length, attached near anterior end of spore. Resting spores, consistently with a companion cell, spherical to oval, 17 to 24 μ in longest diameter, containing one large oil globule surrounded by vacuolate protoplasm. Exospore wall with curved spines up to 5 μ in length. Companion cell spherical or oval, 14 to 20 μ , smooth or with short, closely set spines.

Collected on *Pythium* sp. from Chapel Hill, North Carolina, October, 1940.

OLPIDIOPSIS brevispinosa sp. nov.²

Zoosporangii 10.68–68.40 μ diametro. Zoosporis biflagellatis. Spora perdurante valde brunnea, globosa vel ovali, 10.68–45.10 μ diametro, cellula adhaerente praedita. Episporio cum spinis usque ad 3.56 μ longis. Cellula adhaerente 7.12–25.5 μ diametro, levi vel pariete spinoso.

Olpidiopsis brevispinosa n. sp.

Zoosporangia one to many in terminal or intercalary swellings of the host hyphae, swellings up to 125 μ in diameter. Zoosporangia 10.68 to 68.40 μ in longest diameter, wall smooth or with short bristles, with one to three exit tubes. Zoospores with numerous oil globules, elongate and somewhat reniform, biflagellate, the flagellae of about equal length, attached near anterior end of the spore. Resting spore consistently with a companion cell, dark brown, spherical to oval, 10.68 to 45.1 μ in longest diameter, spines on exospore wall up to 3.56 μ in length, endospore wall 0.75 to 1.32 μ thick, exospore wall 1.78 to 2.50 μ thick.

Collected December 27, 1941, by Philip Couch from Delhi, Louisiana. Parasitic on *Pythium* sp.

The zoosporangia of both of these species of *Olpidiopsis* are entirely similar so that morphologically the two species can be distinguished only by the differences in ornamentation of the resting bodies. Consistently the spines of the resting body of *O. curvispinosa* are bent with a characteristic flexuous appearance. There are, of course, variations in the length and degree of curvature of the spines but the general effect is quite different from that of the rest-

ing body of *O. brevispinosa*. The spines of the resting body of *O. brevispinosa* likewise vary in length but the average length is never as great as that of the spines in *O. curvispinosa*, nor do the spines curve and intertwine as in *O. curvispinosa*. The maximal size of the resting body of *O. brevispinosa* is almost twice that of *O. curvispinosa* and associated with this difference in size is a higher frequency of resting body formation in *O. brevispinosa*.

McLarty (1941) states that the character of the exospore is not a suitable, fundamental diagnostic character, though it is chiefly on the basis of exospore variations and host range that the species of *Olpidiopsis* have been determined. If, however, differences in exospore characters are accompanied by the limitation of the species in question to different host species, as is true of *O. curvispinosa* and *O. brevispinosa*, the species may be considered as valid until there is found a better means of species differentiation.

It was not possible to identify the species of *Pythium* which were attacked by *O. curvispinosa* and *O. brevispinosa*. The host of *O. curvispinosa* does not form oogonia in culture and the only reproductive structure is a filamentous zoosporangium (fig. 6). The vegetative hyphae vary in diameter from 3.2 to 4.3 μ . The host of *O. brevispinosa* also has filamentous zoosporangia (fig. 14) but in addition this species of *Pythium* produces oogonia (fig. 12 and 13) very readily. The oospores, 15.0 to 17.5 μ in diameter, do not fill the oogonium, which measures 20.0 to 25.5 μ in diameter. The antheridia are declinuous and may number one or more on an oogonium. The oogonia and zoosporangia appear to be quite similar to those of *Pythium gracile* but this *Pythium* form did not parasitize *Spirogyra* when placed in contact with filaments of *Spirogyra*.

HOST RANGE STUDIES.—Shanor (1940) has made observations on the host range of five species of *Olpidiopsis*, one of the species being *O. luxurians*. Similar, but less extensive, studies were made with the four species of *Olpidiopsis* discussed in this paper. The purpose of these studies was to obtain further proof of the distinctness of the four morphologically different species, assuming that the inability to transfer one species of *Olpidiopsis* to the normal host of another species of *Olpidiopsis* is an indication of physiological differences of value in diagnosing the parasite species.

Whether or not a given species of *Olpidiopsis* was able to parasitize a certain species of water mold was determined by the following procedure. A large number of spores of the parasite species were added to a hemp seed culture of the water mold to be tested. The cultures were examined for evidences of parasitism twenty-four and forty-eight hours after inoculation, at which time the young parasite was visible in the swellings of the host if infection of the host had been successful. This procedure in each case was repeated three times with consistent results. The results of these experiments are shown in table 1.

² The Latin descriptions were prepared by Alma Holland Beers.

TABLE 1. Chart of reactions of nine species of water molds to one or more species of *Olpidiopsis*. Infection is indicated by a plus sign while non-susceptibility is recorded with a zero mark. Dots opposite certain of the species of water molds in the columns under the names of the parasite species indicate that tests for parasitism were not made in these instances.

Host species	Parasite species			
	<i>O. curvispinosa</i>	<i>O. brevispinosa</i>	<i>O. gracile</i>	<i>O. Aphanomyces</i>
<i>Pythium torulosum</i>	+	0
<i>P. pulchrum</i>	0	0
<i>P. proliferum</i>	0	0
<i>P. sp.</i> (host of <i>O. curvispinosa</i>)	Host	0	0
<i>P. sp.</i> (host of <i>O. brevispinosa</i>)	0	Host	0
<i>P. rostratum</i>	0	0	Host
<i>Aphanomyces cladogamous</i>	Host
<i>A. laevis</i>	0
<i>Plectospora myriandra</i>	0	+

Olpidiopsis curvispinosa was the only parasite which infected a species of *Pythium* (*P. torulosum*) other than its normal host. *Olpidiopsis brevispinosa* did not infect either the host of *O. curvispinosa* or *O. gracile*. Likewise, of all the species of *Pythium* tested with *Olpidiopsis gracile* only its host species, *P. rostratum*, was susceptible. The results obtained with *Olpidiopsis Aphanomyces* were very interesting. The form of *Aphanomyces laevis*, which is the host of the *Olpidiopsis luxurians* studied by Shanor in this laboratory, was found to be unsusceptible to *O. Aphanomyces*. The infection of *Plectospora myriandra*, a water mold which looks like *Pythium* but discharges its spores as in *Aphanomyces*, might indicate a closer relationship of *Plectospora* to *Aphanomyces* than to *Pythium*.

SUMMARY

Pseudolpidium gracile on *Pythium rostratum* was studied, and Butler's observation that the resting body lacks a companion cell is confirmed. A species of *Olpidiopsis*, the resting body of which is without a companion cell was found on *Aphanomyces cladogamous* and is reported as *Olpidiopsis Aphanomyces* Cornu. Two new species of *Olpidiopsis*, *O. curvispinosa* and *O. brevispinosa*, parasitic on two different species of *Pythium*, are described. It was found that it was impossible to transfer any species of *Olpidiopsis* from the host species on which it was collected to the host of any other of the four species.

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TOTAL AUXIN EXTRACTION FROM WHEAT¹

G. S. Avery, Jr., J. Berger and B. Shalucha

A PREVIOUS report from this laboratory (Avery, Berger and Shalucha, 1941) described in some detail the conditions affecting the maximum amount of auxin extractable from maize endosperm. Since wheat, like maize, gave much higher auxin yields when alkaline aqueous extracts were autoclaved a few minutes, it appeared that wheat contained an auxin precursor similar to the one reported for maize. The auxin made available by hydrolysis of the precursor in maize endosperm was reported to be relatively stable to heating with strong alkali (Avery, Berger and Shalucha, 1940) but unstable to heating with strong acid, thus suggesting indoleacetic acid.

Contrary to certain of these findings, high temperature aqueous extraction of auxin from wheat has been reported to give yields inferior to those obtained in low temperature extraction, losses occurring above 60°C. (Haagen-Smit, Leech and Bergren, 1941).² Yields were also reported to be decreased markedly when suspensions were heated at pH values higher than 10.5.

In the light of the foregoing we were led to examine the conditions affecting maximum auxin yields from wheat, particularly those involving temperature, time, and pH. Wheats of high and low protein content were included in the study to determine the effect of this variable on auxin yields, so that conclusions might be generalized as much as possible.

MATERIALS AND METHODS.—All auxin tests here reported are on wheats obtained from the United States Department of Agriculture, through the courtesy of Dr. M. A. McCall. The wheat samples are listed in table 1.

An American strain of Victory Oats grown by Marshall C. Rumsey, Batavia, New York, was used

¹ Received for publication May 14, 1942.

² While this article was in press, another paper appeared concerning auxin extraction from wheat (Haagen-Smit, *et al.*, Amer. Jour. Bot. 29: 500-506, 1942). The conclusions are essentially the same as those in the 1941 paper by the same authors. In view of the similarity in yields obtained by their 48-hour extraction method, and the 15-minute high temperature hydrolysis procedure reported in this paper, it would seem that the latter is to be preferred. The latter method has the advantage of making possible immediate assays, and is not as dependent upon a critical extraction time, *i.e.*, they report increasing yields up to 48 hours, and a marked decrease thereafter.

for all *Avena* tests, and the deseeded test method (Skoog), used for a number of years in this laboratory (Avery, Creighton and Hock, 1939), was employed for all auxin assays. At least two dozen test plants were used per assay, thus making possible calculations of yields which were in every instance from curvatures shown to be in the proportionality range, and close to 10°. After treatments involving pH control or autoclaving with strong alkali, suspensions of the ground wheat kernels were readjusted to approximately pH 6 before centrifuging, dilution and *Avena* assay. Auxin yields are expressed in total degrees *Avena* curvature (TDC), 100,000 TDC equalling *ca.* 1 microgram (μ g.) of indoleacetic acid. Differences of 20 per cent or less on auxin yield data for any one sample are considered to be within the outside limits of experimental variation.

Wherever temperatures of 120°C. are indicated, they were obtained by autoclaving at fifteen lbs. pressure. Control of pH was accomplished by standard buffer preparations, and with a glass electrode. The pH values reported represent the pH of the wheat suspension at the end of the extraction period. However, suitable buffer mixtures were used in all cases to maintain the pH during extraction at the point desired. In some instances, especially the forty-five-hour periods, it was also necessary to add acid or alkali at intervals to help maintain the desired pH.

OBSERVATIONS.—*Effect of temperature.* The procedure of Haagen-Smit, Leech and Bergren was used as reported; in addition, three other procedures were employed involving variations in temperature and time. Following their directions, dry whole wheat grains were soaked for four hours in a sodium hydroxide solution at pH 10.5, then ground in a glass mortar with carefully washed sand, and allowed to stand at 22°C. for forty-five hours (with 1 cc. toluene) with pH maintained at 10.5. The mixture was then centrifuged and the supernatant liquid assayed by the *Avena* test. Table 2 gives the results obtained from such experiments with a high and a low protein wheat.

It is clear from table 2 that there is no appreciable difference in auxin yields between long-period low-temperature and short or long high-temperature extractions, nor was the yield affected by soaking

TABLE 1.

Sample number	Variety	Origin	Per cent protein
1	White Federation, #30332	Pullman, Washington	13.3
2	White Federation, #30333	Pullman, Washington	12.4
3	White Federation, #30347	Bozeman, Montana	9.1
4	White Federation, #30358	Mesa, Arizona	8.1
5	Marquis, #30331	Pullman, Washington	14.3
6	Marquis, #30336	Pendleton, Oregon	10.7

TABLE 2. *Effect of temperature of extraction on auxin yield from wheat. The values given in this and subsequent tables are the averages of two or more assays, each of which was carried out with two dozen Avena test plants; all curvatures used in calculations of yields were in the proportionality range.*

Sample tested	pH during extraction	Auxin yield in 100 thousands of TDC (or micrograms indoleacetic acid) per gram air-dry seeds			
		22°C.		120°C.	
		15 min. ^a	45 hrs. ^b	15 min. ^a	45 hrs. ^a
1	10.5	1.1 ^c	1.9	1.8	2.2
3	10.5	0.7	2.9	3.2	2.6

^a Weighed samples of ground wheat kernels were suspended in M/10 borate buffer, pH 10.5, and treated as indicated. When necessary, pH was maintained at 10.5 by additions of 1.0 N NaOH. Suspensions were neutralized, centrifuged and assayed as usual.

^b These extractions were carried out according to the method of Haagen-Smit, *et al.*; see text.

^c The following is a sample calculation: with an average *Avena* curvature of 11.0° at a dilution of 1 gm. ground wheat in 100 cc. of 1.5 per cent agar-mixture, the yield, substituting in the formula, TDC/gm. =

$$\frac{\text{degrees curvature} \times \text{dilution} \times 100}{\text{weight in gms. of sample}}, \text{ is}$$

$$\frac{11 \times 100 \times 100}{1} = 110,000 \text{ TDC.}$$

Since 100,000 TDC = ca. 1.0 µg. indoleacetic acid, the yield is equivalent to 1.1 µg. of the latter.

whole grains before grinding. For the remaining experiments, therefore, batches of each of the wheat samples were ground finely and used as a uniform source of material.

Effect of time of hydrolysis.—The object of this experiment was to determine the auxin yields from wheats of different protein content, as influenced by heating at 120°C. and pH 9.3 for fifteen and sixty minutes. The data in table 3 indicate that auxin yield is unrelated to protein content of wheat. Moreover, extracts of wheats made at high temperatures show little or no differences in yield whether heated for fifteen or sixty minutes, thus giving further support

TABLE 3. *Effect of time of hydrolysis on auxin yields from wheats of varying protein content. Temperature 120°C., pH 9.3.*

Sample tested	Per cent protein	Auxin yield in 100 thousands of TDC (or micrograms indoleacetic acid) per gram air-dry seeds	
		Time of autoclaving	
		15 minutes	60 minutes
1	13.3	1.9	1.8
2	12.4	1.3	1.4
3	9.1	3.6	3.2
4	8.1	1.8	1.8
5	14.3	0.5	0.6
6	10.7	0.6	0.5

to evidence presented in table 1, namely, that there is no significant loss of auxin at higher temperatures.

Effect of pH of extraction.—For this study low temperature forty-five-hour extraction was employed so that the results might be compared with those in the literature. The results are given in table 4. The data show very low yields at acid pH values,

TABLE 4. *Effect of pH of extraction on auxin yields from wheat. Temperature 22°C., time forty-five hours.*

Sample tested	Auxin yield in 100 thousands of TDC (or micrograms indoleacetic acid) per gram air-dry seeds			
	pH			
	4.3	7.0	10.5	11.5
1	<0.04 ^a	0.5	1.9	1.5
2	<0.04 ^a	0.4	1.3	0.9
3	<0.04 ^a	0.6	2.9	2.0
4	<0.04 ^a	0.2	2.1	1.2
5	<0.04 ^a	0.1	0.7	0.2
6	<0.04 ^a	<0.08 ^a	0.7	0.4

^a Because of their glutinous nature, more concentrated extracts cannot be tested readily, hence the yields are as low or lower than indicated.

but these increase to a maximum at pH 10.5. At pH 11.5 the yields are only about 0.3 to 0.8 times those at pH 10.5, but are two to six times as high as at pH 7. These results differ from those of Haagen-Smit, *et al.*, in that their yield at pH 7 was 1.5 times as great as at pH 11.5. In general then, while the results obtained differ quantitatively in several details from those of Haagen-Smit, *et al.*, they agree in two main principles; namely, that mild hydrolysis with alkali increases the auxin yield, and that high alkalinity causes destruction.

The increases and decreases in auxin yield upon extraction at different pHs, appear unrelated to protein content of the samples assayed.

In maize auxin extractions (Avery, Berger and Shalucha, 1941) the drop in yield between pH 10.5 and 11.5 approximates only 15 per cent. However, the auxin in wheat, from the data in table 4, appeared to be more labile in the presence of alkali, and this lability was studied further.

Instability of wheat auxin in the presence of alkali.—It may be seen from table 5 that the yields on autoclaving with 0.1 N NaOH are appreciably smaller than the maxima obtained at pH 10.5 (table 4), while with 1 N NaOH the yields are not more than 15 to 37 per cent of the maxima. The one exception is sample no. 3, in which the yield is about 70 per cent of the maximum.

The destruction of wheat auxin upon treatment with alkali is in sharp contrast to the alkali-stability of pure indoleacetic acid, and the largest portion of the auxin from maize endosperm; 82 per cent of the latter was stable to autoclaving in 2.5 N NaOH for fifteen minutes. Separate tests have shown that pure indoleacetic acid autoclaved fifteen minutes in 1 N NaOH, in the presence of wheat, is completely

TABLE 5. *Effect of 0.1 N and 1.0 N alkali on auxin yield from wheat. Temperature 120°C., time fifteen minutes.*

Sample tested	Auxin yield in 100 thousands of TDC (or micrograms indoleacetic acid) per gram air-dry seeds	
	0.1 N NaOH	1.0 N NaOH
1	0.8	0.7
2	0.9	<0.2 ^a
3	2.6	2.0
4	0.8	0.5
5	0.3	0.2
6	<0.2 ^a	0.15

^a Because of their glutinous nature, more concentrated extracts cannot be tested readily, hence the yields are as low as or lower than indicated.

stable thus indicating the absence of destructive catalysts.

The data on alkali-instability of auxin from five of the six wheats (tables 4 and 5) suggest that in these samples, an auxin other than indoleacetic acid accounts for 70 per cent or more of the total auxin present. There is no evidence of correlation between the amounts of alkali-stable or alkali-labile auxin and protein content.

Evidence for the existence of two auxin precursors.—To demonstrate the existence of at least one water-soluble precursor, the following experiment was done. A 0.5 gm. portion of wheat sample no. 3 was suspended in 10 cc. of water at pH 5.9 for fifteen minutes, at 22°C. The suspension was then clarified by both centrifuging and filtering. One aliquot of this clarified extract was assayed for auxin without further treatment. A second aliquot was autoclaved for fifteen minutes with an equal volume of M/10 borate buffer, pH 9.6. The auxin content of the unheated extract was found to be 17,000 TDC per gram, while the heated extract gave 94,000. These data are the average of four closely agreeing experiments. This increase of over five-fold demon-

strates the existence of a water-soluble precursor which is converted into auxin upon heating at an alkaline pH. However, it is apparent that not all the auxin precursor of this wheat is readily soluble, since a total yield of 300,000 TDC per gram (vs. 94,000) could be obtained from *suspensions* autoclaved at an alkaline pH.

Some of the data already presented, upon closer analysis, point to the conclusion that at least two precursors exist in certain of the wheat samples. One of these is a precursor of an alkali-stable auxin (possibly indoleacetic acid), the other, a precursor of an alkali-labile auxin. In table 6, figures are given for the minimal amounts of these precursors, calculated from three sets of experimental data.

The yields at pH 10.5 (table 4) represent the total auxin (free auxin plus auxin precursor) obtainable; they agree well with the total auxin yields obtained by autoclaving at pH 9.3, except in the case of sample no. 3.

We may assume that the yields after autoclaving in 1.0 N NaOH (tables 5 and 6) represent alkali-stable auxin, since in at least two cases (samples 3 and 5) yields do not decrease with increasing alkalinity. This auxin may be indoleacetic acid, since the latter is completely stable to such alkali treatment, whether in the presence or absence of wheat. The stability of indoleacetic acid to alkali treatment in the presence of this plant tissue confirms previous experiments on stability of indoleacetic acid to alkali upon autoclaving in the presence of corn meal (Avery, Berger and Shalucha, 1941).

The yields at pH 7 (table 4) may be considered the maximal amounts of free auxin. Separate extractions of wheat samples with ethanol or purified ethyl ether give lower yields of free auxin.³

³ Ethanol, twenty-four-hour extraction, gave 3.6 and 6.0 thousand TDC per gram for samples 1 and 3 respectively; ether, twenty-four-hour extraction, gave 6.0 and 8.6 thousand TDC per gram for samples 1 and 3. These extractions were all made with three changes of solvent in the twenty-four-hour period.

TABLE 6. *Evidence for the occurrence of precursors of two different auxins.*

Treatment	Auxin yield in 100 thousands of TDC (or micrograms indoleacetic acid) per gram air-dry seeds					
	Sample					
	1	2	3	4	5	6
pH 7						
(free auxin yield, from table 4).....	0.5	0.4	0.6	0.2	0.1	<0.08
pH 10.5						
(total auxin [free + precursor] yield, from table 4).....	1.9	1.3	2.9	2.1	0.7	0.7
pH 11.5						
(from table 4).....	1.5	0.9	2.0	1.2	0.2	0.4
N/10 NaOH						
(from table 5).....	0.8	0.9	2.6	0.8	0.3	<0.2
N/1 NaOH						
(alkali-stable auxin, from table 5).....	0.7	<0.2	2.0	0.5	0.2	0.15
Precursor of alkali-stable auxin.....	0.2	<0.2	1.4	0.3	0.1	<0.07
Precursor of alkali-labile auxin.....	0.7	0.7	0.3	1.4	0.4	0.47

As an example of the calculations, let us consider sample no. 3. This wheat contains 2.0 μ g. indoleacetic acid equivalents of an alkali-stable auxin, and at most, 0.6 μ g. of this are present in a free form before hydrolysis with alkali, i.e., at least 1.4 μ g. of a precursor were hydrolyzed to produce an alkali-stable auxin. Of the 2.9 μ g. of total auxin obtainable, 0.9 μ g. was destroyed by 1.0 N NaOH. At most, 0.6 μ g. of free auxin was alkali-sensitive; therefore, at least 0.3 μ g. of a precursor was present which on mild hydrolysis with alkali produced an alkali-sensitive auxin. It is obvious, therefore, that two auxins differing markedly in their alkali-stability are formed upon mild hydrolysis with alkali. That these two auxins are derived from different precursors is a logical conclusion.

Preliminary work on the chemical nature of the free auxin in wheat no. 3 indicates that some of it is indoleacetic acid or an indole derivative. A purified fraction obtained from 4 kg. of wheat gave the Winkler color test for indoleacetic acid, with an intensity approximately proportional to the concentration of acid calculated to be present from the *Avena* assay of the same fraction (1.2 per cent pure indoleacetic on dry weight basis).

While the above results are considered to be clearly indicative of the presence of two precursors, it is understood that the evidence does not constitute conclusive proof.

Additional evidence for the existence of two different auxin precursors in higher plants already exists in the literature. It has been suggested (Went and Thimann, 1937, p. 65) that the increased auxin yields obtained on saponification of maize oil were due to hydrolysis of an ester type of auxin a precursor. It has also been shown that in maize endosperm 90 per cent of the total auxin obtainable is present as a precursor of indoleacetic acid (Avery, Berger and Shalucha, 1941). The maize kernel, therefore, as well as the wheat kernel, apparently contains at least two different auxins, as well as two different precursors.

DISCUSSION.—The highest yields obtained in this study are approximately half those reported by Haagen-Smit, *et al.* This dissimilarity in yield might be explained in part by differences in wheat samples used; also in part by differences in details of the technique of assay.

Indoleacetic acid was shown by Haagen-Smit, *et al.* (1941), to be present in maize, but no evidence was presented as to the chemical nature of auxin in wheat. Since we have found marked differences in the alkali-stability of the auxins from maize and wheat, we are studying the chemical nature of wheat auxin. Kögl, *et al.*, distinguished stability in the presence of alkali as a characteristic of indoleacetic acid, and reported the isolation of this compound from yeast, human urine, etc. (cf. Boysen Jensen, Avery and Burkholder, 1936). Since 1934 there have been few advances in the chemical identification of auxins extracted from plant material, but there has been a gratifying increase in the number of studies

reporting at least presumptive evidence for the presence of indoleacetic acid in higher plants: Lefèvre (1938), using color tests, etc., reported that radish, cauliflower, water cress and asparagus contained indoleacetic acid and related acids. Riker (1939) reported that "growth substances similar to beta-indole-acetic acid in their reaction to hot acid and alkali have been obtained by ether extraction from" . . . normal and pathological tomato tissue. Van Overbeek (1940) tested extracts of the brown alga *Macrocystis*, and found that "invariably the auxin present in it was destroyed by acid, but not by alkali, indicating that indoleacetic acid was present"; evidence from determinations of the diffusion coefficient supported this conclusion. Link, *et al.* (1940), studying the auxin content of certain aphids, and the host plants on which they lived, found both acid- and alkali-stable auxins. Avery, Berger and Shalucha (1940, 1941) presented strong evidence that 90 per cent of the total auxin in maize endosperm was probably indoleacetic acid, and this conclusion was independently confirmed by Haagen-Smit, *et al.*, who isolated indoleacetic acid from maize. The question now pressing for solution is the chemical nature of the approximately 70 per cent or more of the total wheat auxin which is not indoleacetic acid.

It remains to be demonstrated whether either of the auxin precursors in wheat is identical with that previously established for maize.

SUMMARY AND CONCLUSIONS

Total auxin yields from high and low protein wheats are of approximately the same magnitude whether samples are extracted for forty-five hours at 22°C., or for fifteen minutes at 120°C., i.e., a higher temperature during the extraction period greatly decreases the time required without decreasing the yield.

The highest yields are obtained when extractions are carried out at pH 9.3 to 10.5. Lower yields are obtained at pH 4, 7 and 11.5, but the yield at 11.5 is greater than at pH 7. Marked destruction of the auxin from five out of six wheat samples tested occurs upon fifteen-minute autoclaving with 1.0 N NaOH, in contrast to the alkali-stable auxin present in maize endosperm; thus 70 per cent or more of the total auxin is a compound other than indoleacetic acid.

Auxin yields are apparently unrelated to protein content.

Auxin yields from wheat samples of different protein content differ in magnitude as much as four-fold.

The probable presence is established of two precursors which are converted into two auxins upon hydrolysis at pH 10.5. These auxins differ markedly in their stability in the presence of alkali.

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A NEW CHYTRID WITH GIANT ZOOSPORES: SEPTOCHYTRIUM MACROSPORUM SP. NOV.¹

John S. Karling

IN CONTINUATION of a survey begun in 1940 of the chytrid flora of the eastern United States the author made further extensive collections of water samples containing vegetable debris during a tour of the south and southwest in the late summer of 1941. Samples of water from roadside ditches, ponds, lakes, swamps, and flowing streams were collected in pint-size thermos bottles and baited with bleached fragments of young corn leaves, cellophane, and bits of Kleenex cleansing tissue. Pieces of ice were added to the bottles from time to time so that the samples were kept at temperatures varying from 38° to 45°F. By such means it was possible to prevent too rapid development of the fungi present during transportation from one locality to another and until time and better facilities were available for microscopic study. The samples of water thus collected and preserved were eventually brought to Columbia University, re-baited, and studied in the manner described by the author in previous publications (1941).

In collections from Arkansas an unusually large chytrid occurred which appears to be new. It was first found in a water sample taken from a roadside ditch near Forest City, Arkansas, which had been baited with Kleenex cleansing tissue. By the time this sample reached New York City the rhizomycelium of the fungus had completely ramified the tissue and formed a dense network of hyphae, rhizoids, and zoosporangia among the cellulose fibers. The same species was next collected in a cyprus swamp near Little Rock, Arkansas, and since that time it has also been found in Texas, Louisiana, Mississippi, Alabama, Florida, Georgia, South and North Carolina, Virginia, and Tennessee. It has also been collected recently near Greenwood Lake, New Jersey, which indicates that it has a very wide distribution.

This species is characterized by an unusually coarse rhizomycelium, operculate zoosporangia, and

giant zoospores. Apart from the absence of pseudo-septa or trabeculae in the tenuous portions of the rhizomycelium, its structure, development, and organization are fundamentally the same as those of the genus *Septochytrium*. It is accordingly included in this genus for the time being and named *S. macrosporum* sp. nov. because of its unusually large zoospores.

SEPTOCHYTRIUM macrosporum sp. nov.—Fungus saprophyticus; thallo praecipue polycentrico, rhizomycelio, 5-15 μ dia.; zoosporangiis hyalinis, laevis, globosis, 15-280 μ , pyriformibus, 30-50 \times 70-190 μ , fusiformibus, irregularibus; operculatis 9-16 μ dia. Zoosporis hyalinis, globosis, 11-13 μ dia. Sporis perdurantibus laevis, verruciformibus, luteo-brunneis, globosis, 25-115 μ , ovatis, 30-50 \times 40-75 μ .

Septochytrium macrosporum sp. nov.—Thallus predominantly polycentric, occasionally monocentric. Rhizomycelium unusually coarse, richly branched, rarely septate, with thick walls which stain brick-red to reddish-lavender with chloro-iodide of zinc; tenuous portions apart from rhizoids 5 to 15 μ in diam.; rhizoids numerous and richly branched; intercalary enlargements broadly or narrowly spindle-shaped, elongate, fusiform, and irregular. Zoosporangia terminal or intercalary, delimited from remainder of thallus by cross septa, non-apophysate, spherical, 15-280 μ , pyriform, 30-50 μ \times 70-190 μ , obpyriform, broadly fusiform, obclavate, utriform, and sometimes irregular; hyaline and smooth; opercula 9-16 μ in diam. Zoospores spherical, 11-13 μ , average 12.2 μ , with one larger, 2.5-3.5 μ , and three to six minute refractive globules; emerging fully formed and lying quiescent for a few moments in a globular mass at the exit orifice before swimming away; intermittently amoeboid. Resting spores intercalary, oval, 30-50 \times 50-75 μ , spherical, 25-115 μ , amber, and yellow to light-brown in color, smooth or covered with coarse, simple or branched

¹ Received for publication May 15, 1942.

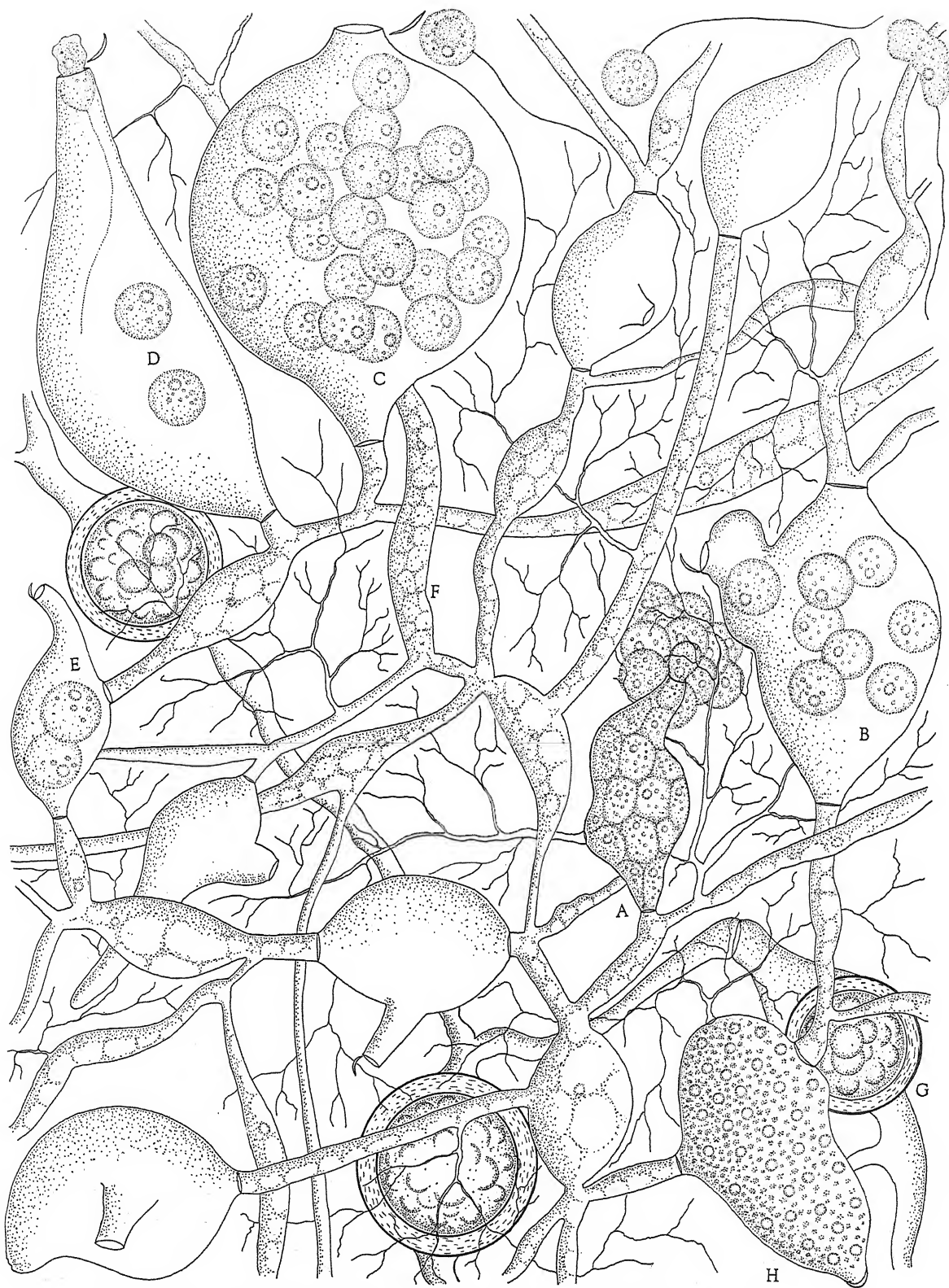


Fig. 1. Portion of the rhizomycelium of *S. macrosporum* growing in Kleenex cleansing tissue.

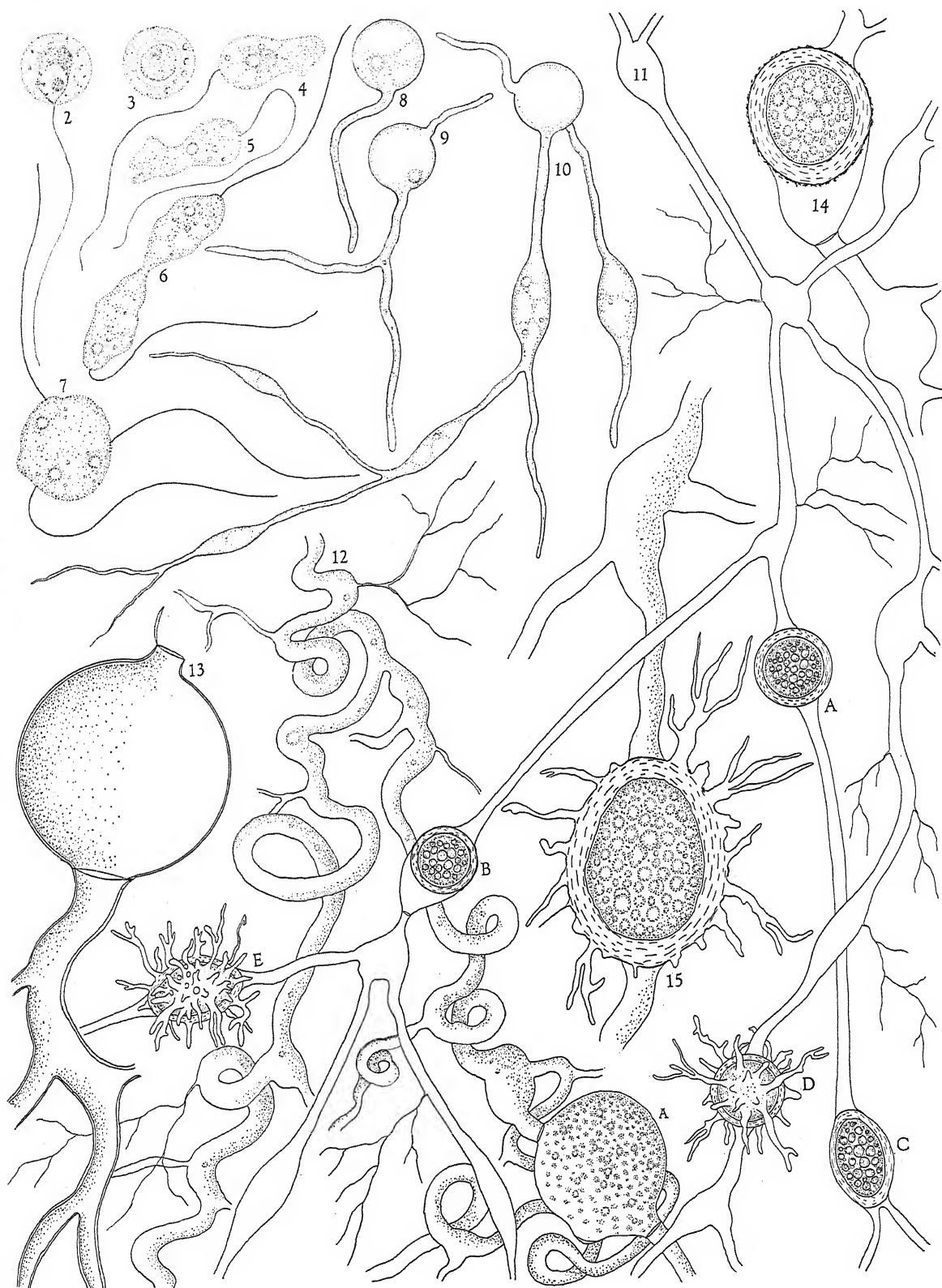


Fig. 2-15.—Fig. 2. Zoospore fixed and stained *in toto*.—Fig. 3. Posterior view of zoospore.—Fig. 4, 5. Amoeboid zoospores.—Fig. 6. Biflagellate zoospore dividing.—Fig. 7. Giant triflagellate zoospore.—Fig. 8, 9, 10. Germination of zoospores.—Fig. 11. Elongate sparsely branched rhizomycelium with five resting spores growing in cellophane.—Fig. 12.

pegs or filamentous extensions, 4–18 μ long; wall 4–6 μ thick, content coarsely granular with one to several large refractive granules; germination unknown.

Saprophytic in vegetable debris in ponds, brooks, lakes, swamps, etc., in Arkansas, Texas, Louisiana, Mississippi, Alabama, Florida, Georgia, Tennessee, South and North Carolina, Virginia, and New Jersey, U. S. A.

In diameter of rhizomycelium and extent of growth as well as zoospore size, *S. macrosporum* is the largest of all known polycentric chytrids. Apart from the spindle-shaped intercalary swellings which are usually quite large, the tenuous portions of the rhizomycelium may, in exceptional cases, attain a diameter of 15 μ (fig. 1F), and on cellophane single thalli have been found to extend over a distance of 3.5 mm. In addition, the zoosporangia may sometimes be almost 300 μ in diameter. While such large thalli are exceptional, they nevertheless indicate the remarkable size which this species may attain. Figure 1, except the resting spores, is a drawing of a portion of the rhizomycelium in a piece of cleansing tissue from the original material collected at Forest City, Arkansas, and illustrates quite well the extent of branching and richness of thallus development. In cellophane and corn leaves, however, the frequency of branching may be considerably less, so that long tubular unbranched filaments up to 200 μ in length may sometimes be found (fig. 11). However, as the rhizomycelium grows out from pieces of cellophane into the water, its habit of growth changes markedly. It becomes loosely or closely coiled as is shown in figure 12 and rarely follows a straight line. Growth on corn meal and potato dextrose agar is rather slow, but the rhizomycelium branches more frequently than in cellophane. In all types of media and substrata, branching usually occurs most abundantly in the vicinity of the intercalary enlargements, as is shown in figures 1 and 11. Rhizoids are usually abundant and may arise from the surface of sporangia and intercalary enlargements (fig. 1, 11) as well as from the tenuous portions of the rhizomycelium. Well-defined cross walls delimit the zoosporangia and resting spores, but no septa have been found in other parts of the thallus. No constrictions in the filaments nor bands nor trabeculae of opaque material which extend partly or completely across filaments have been observed in this species. The lack of these characters distinguishes the rhizomycelium of *S. macrosporum* sharply from that of the type species, *S. variabile*, described by Miss Berdan (1939, 1941). As noted elsewhere, the walls of young thalli stain brick-red or reddish-lavender when tested with chloro-iodide of zinc. In large old thalli the walls usually become thick (0.7–1.8 μ) and rigid, and withstand disintegration for several months.

While the thallus of *S. macrosporum* is predominantly polycentric, it may often be monocentric in

small reduced thalli (fig. 13). In such instances, it is strikingly similar to the thallus of *Endochytrium operculatum* and may be easily mistaken for the latter species. Such thalli may be monorhizoidal with the main axis of the absorbing system attached at the base or polyrhizoidal with numerous rhizoids arising from the lower periphery of the sporangia. In these thalli the single sporangium originates from the initial intramatrical swelling on the germ tube.

The zoospores of *S. macrosporum* emerge singly and fully developed from the sporangia and lie inactive for a short while at the exit orifice before swimming away. As a result, a large globular mass of spores accumulates at the apex of the sporangium as is shown in figure 1A. However, this mass usually breaks up before most of the zoospores have emerged, so that a large number of spores are generally to be found in the sporangia. These soon begin swarming within, and as the individual spores reach the exit orifice they emerge (fig. 1D) and swim directly away without coming to rest. In cover glass mounts the exit orifice may sometimes become closed by pressure or obstruction, and in such cases the trapped zoospores may continue to swarm for many hours before coming to rest within the sporangium. In one case of a sporangium with eight trapped zoospores active motility continued for forty-two hours. Such zoospores eventually become inactive, and it is not uncommon to find sporangia with numerous quiescent zoospores like those shown in figures 1B to 1E.

The zoospores of *S. macrosporum* are larger than those of any known chytrid and also most species of the Blastocladales, Monoblephoridiales, Saprolegniales, and Peronosporales. They nevertheless have the typical chytridiaceous appearance, structure, and type of motility (fig. 2–5). The refractive globule seems quite small in proportion to the size of the spore body, although it ranges from 2.5 to 3.5 μ in diameter. In addition to this globule, the zoospore contains three to six minute ones which impart a slightly greyish granular appearance to the zoospore. Because of the relative minuteness of these refractive globules, the mature zoosporangium with fully delimited spores appears less refractive and more greyish granular than those of *S. variabile*. In fixed and stained preparations, the nucleus of the zoospore, in median longitudinal views, is obpyriform and tapers toward the point of attachment of the flagellum (fig. 2). Enveloping its upper one-third is a densely-stainable lunate body or nuclear cap, of the type described by the author (1937) in *Cladochytrium replicatum*. This body may also become faintly visible in living zoospores toward the close of their motile or amoeboid stage (fig. 4). When observed from the posterior end of the zoospore, the nucleus in living spores often appears to lie in a vacuole as is shown in figure 3. Occasional bi- and triflagellate zoospores (fig. 6, 7) have been

Coiled rhizomycelium growing in water at the edge of a piece of cellophane.—Fig. 13. Monocentric reduced thallus.—Fig. 14. Smooth resting spore in median view.—Fig. 15. Median view of resting spore with warts and branched pegs on its surface.

found, which appear to be the result of incomplete or unequal cleavage. Some of the biflagellate individuals have been seen to constrict and undergo fission (fig. 6). Triflagellate zoospores with a diameter of $18\ \mu$ have occasionally been found.

Germination of the zoospores and the development of the thallus in *S. macrosporum* are fundamentally similar to those of other cladocytriaceous chytrids with intercalary enlargements in the rhizomycelium and need not be described in detail. As is shown in figures 8 to 10, one and sometimes two or three thick germ tubes are formed which penetrate the substratum and begin to branch. A fusiform swelling may develop in the germ tube (fig. 10) before it branches or later. These swellings may remain comparatively small or develop into zoosporangia. In any event, they function as vegetative centers of growth, organization, and reproduction. They do not, however, become uni- or multiseptate as in *Cladocytrium replicatum*, *C. tenue*, etc. Although no intensive study of fixed and stained material has yet been made, it will probably be found that the nuclei are localized in these enlargements as has been shown for *C. replicatum*. The germ tube continues to grow and elongate until an extensive rhizomycelium with numerous rhizoids is established. The intercalary zoosporangia develop from the fusiform swellings, and in the early developmental stages it is impossible to determine whether a sporangium or merely an elongate swelling is to be formed. The terminal zoosporangia, on the other hand, develop by enlargement of the tips of the rhizomycelium.

The protoplasm of the developing rhizomycelium has a characteristic vacuolate and alveolar structure, as is shown in figure 1. The refractive material is often greatly dispersed, so that the protoplasm has a distinct greyish-granular appearance. This is true to some extent of the contents of the zoosporangia also, and as a result the interior of mature sporangia appears less refractive (fig. 1H, 12A) than that of *S. variable*, *Endochytrium operculatum*, etc.

Thick-walled dormant spores have been found only in the collections of material from Greenwood Lake, New Jersey. They were first observed by Miss A. Johansen in cultures kept for several days in a refrigerator at approximately 5° to 10°C ., and the writer is very grateful to her for these data. Whether or not the spores were formed in reaction to the low temperatures is uncertain, since additional experiments along this line have not been successful. So far, only intercalary resting spores have been found (fig. 11A-11E), and these develop in the same manner as the zoosporangia from intercalary swellings. Up to a certain stage the two are indistinguishable, but after that the protoplasm of the incipient resting spores becomes more coarsely granular and refringent. Simultaneous with these changes, the wall begins to thicken, and in mature spores it may attain a thickness of $6\ \mu$. In this respect *S. macrosporum* differs markedly from *S. variable*. The wall appears to consist of more than one layer and is usually yellow, amber, and light brown in color with a faint

tinge of green. In the smooth spores its outer periphery may often appear slightly incrustated (fig. 14) as if material from the substratum had condensed on its surface. The content of most spores is coarsely granular, and in only two instances so far have spores with one large refringent globule been found. In a few cases the spores only partially filled a thin-walled vesicle (fig. 1G, 11B, 14), which suggests that the contents of the incipient spores contracted and became invested with a thick wall, leaving the remainder of the original vesicle empty. That such structures are to be interpreted as rudimentary oogonia with a single oospore is quite unlikely, in the author's opinion.

As noted in the diagnosis above, two types of resting spore occur—smooth ones and others, the outer wall of which is covered with blunt pegs or short, coarse, hypha-like branches (fig. 11D, 11E, 15). Whether the latter are true resting spores or zoosporangia which have encysted and developed thick walls is not altogether certain at present, since all of their developmental stages have not been seen. However, a few incipient thin-walled stages have been found in which the sporangia-like vesicles were covered with numerous short rhizoids, which suggests that the branches shown in figure 15 are rhizoids that have thickened and become contorted. Figure 15 shows a median section of such a spore. Its coarsely granular content and thick wall are strikingly similar to those of the smooth spores. So far, germination of these resting spores has not been observed, but it doubtless occurs in the same manner as in *S. variable*.

In addition to *S. macrosporum*, described above, a large number of well-known chytrids were collected on this survey. The range of these species has thus been greatly extended. Inasmuch as no extensive collections of saprophytic chytrids have been made previously in many of the southern and southwestern states, the species collected on this survey are listed according to the states visited.

ARKANSAS

Septochytrium macrosporum and *S. variable*. Forest City and Little Rock, Arkansas, 1941.

Cladocytrium replicatum, *C. crassum*, and *C. hyalinum*. Arkadelphia, Brassfield, and Prescott, Arkansas, 1941.

Nowakowskiella profusum, *N. elegans*, *N. hemisphaerospora*. Hope and Sheppard, Arkansas, 1941.

Endochytrium operculatum and *Entophlyctis texana*. Malvern, Arkansas, 1941.

ALABAMA

Catenaria Anguillulae and *Catenochytridium carolineanum*. St. Elmo, Alabama, 1941.

Cladocytrium replicatum, *C. crassum*, and *C. hyalinum*. Mano and Irvington, Alabama, 1941.

Endochytrium operculatum and *E. texana*. Loxley, Alabama, 1941.

Nephrochytrium aurantium, *Nowakowskiella profusum*, *N. elegans*, *N. ramosum*, *N. hemisphaero-*

spora, *Septochytrium macrosporum*, *S. variable*, and *Septochytrium* sp. Grand Bay, Alabama, 1941.

FLORIDA

Catenochytridium carolineanum, *Cladochytrium replicatum*, *C. crassum*, *C. hyalinum*, *Cylindrochytrium Johnstonii*, *N. profusum*, and *N. elegans*. Milton, Florida, 1941.

Nowakowskiella ramosum and *N. hemisphaerospora*. Tallahassee, Silver Springs, Funiak Springs, Marineland, and St. Augustine, Florida, 1941.

Septochytrium macrosporum and *S. variable*. Lake George, Florida, 1941.

Endochytrium operculatum and *E. digitatum* (?). Silver Springs, Florida, 1941.

Rozella Cladochytrii on *N. profusum*, *N. elegans*, and *S. macrosporum*. Marineland, Florida, 1941.

GEORGIA

Catenochytridium carolineanum, *Cladochytrium replicatum*, *C. hyalinum*, *C. crassum*, *Endochytrium operculatum*, *Nowakowskiella elegans*, *N. profusum*, *N. hemisphaerospora*, *Septochytrium macrosporum*, and *S. variable*. Seals, New Brunswick, and Savannah, Georgia, 1941.

LOUISIANA

Catenaria Anguillulae, *Catenochytridium carolineanum*, *Cladochytrium replicatum*, *C. crassum*, *C. hyalinum*, *Diplophlyctis intestina*, *Endochytrium operculatum*, *Entophlyctis texana*, *E. heliomorpha*, *Nowakowskiella profusum*, *N. elegans*, *N. ramosum*, and *N. hemisphaerospora*. Lake Charles, Louisiana, 1941.

Septochytrium macrosporum and *S. variable*. Franklin, Louisiana, 1941.

MISSISSIPPI

Catenaria Anguillulae, *Catenochytridium carolineanum*, *Cladochytrium replicatum*, *C. hyalinum*, *Endochytrium operculatum*, *Entophlyctis texana*, *N. profusum*, *N. hemisphaerospora*, and *S. macrosporum*. Christian Pass, Mississippi, 1941.

NORTH CAROLINA

Catenochytridium carolineanum, *Cladochytrium hyalinum*, *Diplophlyctis intestina*, *Endochytrium operculatum*, *Entophlyctis texana*, *E. heliomorpha*, *Nephrochytrium aurantium*, *Rhizophlyctis peterseii*, *Septochytrium macrosporum*, and *S. variable*. Great Smokies National Park, North Carolina, 1941.

SOUTH CAROLINA

Catenochytridium carolineanum, *Cladochytrium crassum*, *C. replicatum*, *C. hyalinum*, *Cylindrochytridium johnstonii*, *Diplophlyctis intestina*, *Endochytrium operculatum*, *Entophlyctis texana*, *E. heliomorpha*, *Nephrochytrium aurantium*, *Nowakowskiella elegans*, *N. profusum*, *N. hemisphaerospora*, *Rozella Cladochytrii* on *N. profusum*, *Septo-*

chytrium macrosporum and *S. variable*. Cyprus Gardens, Charleston, and Wolfboro, South Carolina, 1941.

TENNESSEE

Cladochytrium crassum, *C. hyalinum*, *N. profusum*, and *N. elegans*. Knoxville, Tennessee, 1941.

Nowakowskiella hemisphaerospora, *S. macrosporum*, and *S. variable*. Nashville, Tennessee, 1941.

TEXAS

In addition to the species reported previously (1941b) from Texas, *Nowakowskiella hemisphaerospora*, *Septochytrium macrosporum*, *S. variable*, and *Nephrochytrium aurantium* were collected at Hamilton Pool on the Pedernales River and at Barton Springs, Austin, Texas.

VIRGINIA

Septochytrium macrosporum and a large saprophytic *Diplophlyctis*-like species were collected in the swamps near the Pamunky River, New Kent County, January, 1942, in addition to the chytrids listed from this region in 1941. The *Diplophlyctis*-like species is considerably larger in sporangium and resting spore size than either *D. intestina* or *D. laevis* and may possibly be a new species. It is now under investigation at Columbia University.

This brief and incomplete survey of chytrids in the southern and two southwestern states shows that the large saprophytic species are well distributed, and as common in occurrence as other aquatic fungi. Particularly noteworthy is the wide distribution of species of *Cladochytrium*, *Endochytrium*, *Nowakowskiella*, and *Septochytrium*. *Diplophlyctis intestina* and *E. heliomorpha* were present wherever species of *Nitella* and *Chara* were collected. *Nowakowskiella ramosum*, which had hitherto been reported only once from the New World (Karling, 1941b), was found in two new localities, Alabama and Florida, while *N. hemisphaerospora* proved to be as widely distributed as *N. elegans* and *N. profusum*. *Nowakowskiella hemisphaerospora* was first collected by the writer in Virginia in 1938 and kept in culture since that time, but no opportunity was available to complete a study of its life cycle. It has recently been fully described and diagnosed as a new species by Shanor (1942), who found it in Illinois. The present survey indicates that it is very common in occurrence. The thalli of what was reported as *C. carolineanum* from Cyprus Garden, South Carolina, were nearly all elongate, cylindrical and tubular and strikingly similar to those of *Cylindrochytridium Johnstonii*, except for the lack of a septum and other differences in the compound apophysis. Whether this isolate is a new species, a strain, or variety of *C. carolineanum*, or merely represents variations of the latter species is now being determined from mono-zoospore cultures. Its similarity in shape, appearance and structure nevertheless suggest a close rela-

tionship between *Cylindrochytridium* and *Catenochytridium*.

In addition to *S. variable* and *S. macrosporum*, a third species of this genus was found in Alabama, North Carolina, and South Carolina. It differs chiefly from the other two species by the lack of a conspicuous refractive globule in the zoospores. Instead, they contain numerous minute granules which give the zoospores a characteristic greyish granular appearance. In addition, its rhizomycelium, apart from the sporangia, is wavy and undulating in contour. It lacks the septa or trabeculae of *S. variable* and in general is smaller than *S. macrosporum*. What appears to be the same chytrid has since been collected by Miss A. Johansen in New Jersey and is now under investigation. Resting spores of *Catenaria Anguillulae* were abundant in the material collected in Cyprus Gardens, South Carolina, and this is the first report of their occurrence in the New World. They are spherical, 20–35 μ , oval, 20–30 $\mu \times$ 40–50 μ , oblong, 30 \times 60 μ , full of yellowish-amber refractive material, and have a fairly thick hyaline

wall. As Buckley and Clapham (1929) have shown in England, they are sometimes formed by the contraction and encystment of the contents of incipient zoosporangia. It is, therefore, not uncommon to find resting spores lying in uteriform vesicles which have a long unopened exit tube.

SUMMARY

Septochytrium macrosporum n. sp. was first collected at Forest City, Arkansas, and has since been found in vegetable debris in Alabama, Florida, Georgia, Louisiana, Mississippi, New Jersey, North Carolina, South Carolina, Tennessee, Texas, and Virginia. It is the largest of all known chytrids and is characterized by an unusually coarse and extensive rhizomycelium, giant zoospores, and smooth and warty resting spores. The spherical zoospores range from 10 to 13 μ in diameter and are among the largest of all known aquatic fungi.

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BREEDING WORK TOWARD THE DEVELOPMENT OF A TIMBER TYPE OF BLIGHT-RESISTANT CHESTNUT: REPORT FOR 1941¹

Arthur Harmount Graves

JAPANESE-AMERICAN HYBRIDS.—Our breeding work with the chestnut was started in 1930, with the aim of combining the tall, erect habit of the American tree with the disease resistance of the Japanese species, a tree of comparatively low stature. The ultimate purpose has been to develop a disease resistant chestnut tree of timber type for reforestation purposes, to replace the American chestnut tree, *Castanea dentata*, now practically extinct, at least as a forest tree.

From 1932 to date the germination of the hybrid nuts has been good, and since we have each year continued the breeding of these two species, using

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We are pleased to acknowledge the continued cordial cooperation of the Division of Forest Pathology, U. S. D. A. Also to the many individuals and institutions that have sent us pollen or nuts, or have helped us in other ways, we take pleasure in expressing our appreciation.

many varieties of the Japanese parent, we now have a considerable number (about 150) of F₁ Japanese-American hybrids, ranging in age from one to ten years. Repeated tests by inoculation, by the method described by the writer (1938b), with the causal fungus in these F₁ hybrids show conclusively that they are susceptible to the blight; not, it is true, to such a degree as the American parent is, but sufficient to make them useless for reforestation stock, at least for tall timber trees. However, their erect habit is like that of the American parent; and in many individuals, the annual rate of growth far surpasses that of the American (Graves, 1938a).

THE GENETIC SITUATION.—We have now reached a point where we can sketch roughly the genetic situation in these Japanese-American hybrids with regard to their growth habit and resistance or susceptibility to the blight.

Let us represent the tall, erect growth of the American chestnut (*Castanea dentata*) by T, and its susceptibility to blight by S. Then the comparatively low stature of the Japanese species (*C. cre-*

nata) may be designated by t, and its disease resistance by s.

Accordingly, when *Castanea dentata*, TTSS, is crossed with *Castanea crenata*, ttss, each one of the offspring may be represented by TtSs, where tallness and disease susceptibility are dominant. This, it will be seen, corresponds essentially to the situation as we now find it among our F₁ Japanese-American hybrids.

That the case is, in all probability, not so simple as this, is obvious. For the "tallness" may be a combination of several factors; e.g., annual rate of growth, or, better, relative rapidity of growth, and also growth habit, i.e., direction of growth, the latter depending possibly on the relative quantity (or quality) of growth hormones in terminal and lateral buds, as determining whether the growth shall be orthotropous or plagiotropous. Also, "susceptibility" is, in all likelihood, not the result of a single factor, a partial evidence of which we have in the fact that the F₁ hybrids are more disease resistant than the American parents. Possible factors which combine to produce disease resistance may be the relative osmotic tension of cells, or of particular tissues (which may differ at different periods of the year), relative thickness of cell walls, character of cell contents—qualitative and quantitative—rapidity of formation of new tissues, etc. However, at present, any attempt to state the factors operating is largely a matter of speculation. The fact remains that in the F₁ Japanese-American hybrids the American characteristics are dominant.

FURTHER BREEDING OF THE F₁ JAPANESE-AMERICAN HYBRIDS.—If the case were a simple, bifactorial one, the further breeding of the F₁ hybrids would yield on the average for every sixteen of the progeny: nine tall and susceptible, three tall and resistant, three dwarf and susceptible, and one dwarf and resistant. But, as we said above, the case is obviously not as simple as this. Nevertheless, we know that the proportion of the desired offspring resulting from such further breeding will be greater or lesser, depending on the actual complexity of the genetic constitution. And, furthermore, it is evident that in order to obtain the desirable forms, the breeding must be continued beyond the F₁ stage, either by interbreeding the F₁s, or by backcrossing with disease resistant individuals.

This whole situation was foreseen, or rather suspected, in 1937, at which time we began interbreeding our hybrids, and also backcrossing them, not only with resistant Japanese but with resistant Chinese, with the result that we now have a considerable number of F₂s and a combination of F₁s and resistant Japanese or Chinese in both our own and cooperative plantations (Graves, 1940). Some of these flowered this year (see third starred item in table 1) and the breeding is being continued with them.

A SIMILAR CASE IN TOBACCO BREEDING.—The case of the Japanese-American chestnut hybridization has some points of similarity with the breeding of

certain tobaccos now under way at the Connecticut Agricultural Experiment Station. An account of that work has not yet been published, but I have the permission of Dr. P. J. Anderson of the Tobacco and Vegetable Substation at Windsor, Connecticut, to give a general outline of the work and indicate the results to date. Dr. Anderson (1918) is also well known for his work on the chestnut blight fungus, *Endothia parasitica* (Murr.) P. J. & H. W. Anderson.

The Connecticut Broadleaf tobacco, which has very broad leaves but is susceptible to the mosaic disease, is being bred with a tall, disease resistant

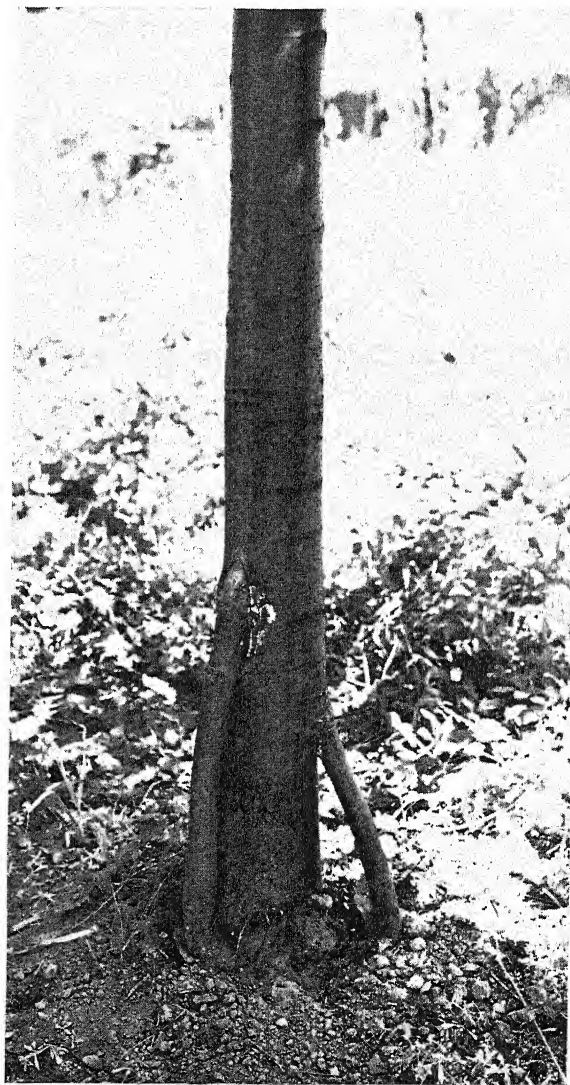


Fig. 1. Japanese-American hybrid chestnut (*Castanea crenata* × *C. dentata*, Hammond, 99A33) eight years old, showing three basal shoots inarched (in spring of 1941) in order to keep tree alive and vigorous for further breeding. Tree is blighted for more than half of circumference at base near ground. Photo by Louis Buhle, September 13, 1941. (Photograph loaned by Brooklyn Botanic Garden.)

variety known as Ambalema, imported from Colombia, South America. In the F_1 generation all plants were susceptible to the mosaic, but in the F_2 generation the expected segregation occurred in such a way as to indicate that disease resistance is a regular recessive character. By selection of desirable individuals and backcrossing, Dr. Anderson says, in correspondence, "I am getting much nearer to the qualification which I require, but have not yet obtained a seed which I am ready to distribute or recommend to the grower. This may take several more years of selection, but I feel sure that it is only a question of perseverance and patience, and the qualities can be definitely obtained." In our chestnut breeding work the progress will naturally be much slower than it is with tobacco where two generations can be obtained each year. In the Japanese-American hybrid chestnuts the generations are usually from three to six years apart.

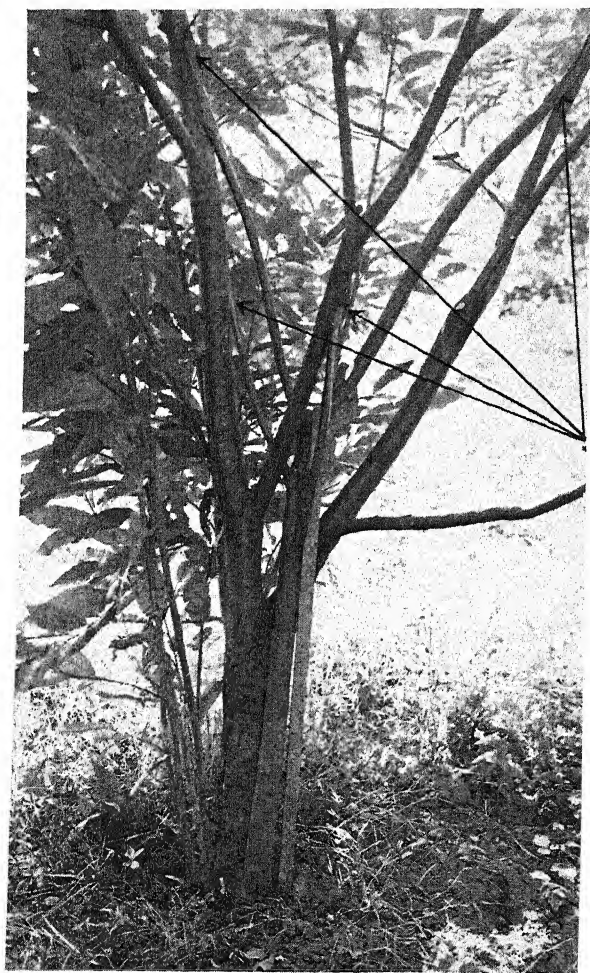


Fig. 2. Japanese-American hybrid chestnut (*Castanea crenata* \times *C. dentata*, Minturn 14-33) showing four inarchings of basal shoots. Tree eight years old, badly blighted near base. Photo by Louis Buhle, September 13, 1941. (Photograph loaned by Brooklyn Botanic Garden.)

METHOD OF PROLONGING THE LIFE OF F_1 JAPANESE-AMERICANS FOR FURTHER BREEDING.—The fact that the F_1 Japanese-American chestnuts ordinarily contract the blight early in life (usually before the age of ten years) makes it difficult to continue breeding with them in order to bring about the segregation above indicated; for, if badly blighted, they become sickly and soon die, at least above ground. Especially is this true when the blight fungus attacks the tree at the base, as often happens. In this case, however, young shoots or "suckers" are usually developed from the root collar at the ground level. It is then a simple matter to graft the tips of these shoots into the trunk above the lesion caused by the blight. In 1941 several trees were rehabilitated in this way. Figure 1 shows a tree that was blighted for more than half of the circumference of the trunk at the base, but by the method above described, three basal shoots were turned into the trunk above, with the result that the tree was vigorous during 1941, was crossed with another F_1 , and bore a good crop of hybrid nuts. Figure 2 shows a tree in which, on account of a somewhat higher location of the lesions, the grafts were made much higher up.

CHINESE-AMERICAN HYBRIDS.—We began crossing the American and Chinese species in 1934, using for the Chinese parents fine specimens that had been given us in 1929 (then two years old) by the Division of Forest Pathology, U. S. D. A. Our inoculation experiments have shown that the Chinese species, *C. mollissima*, is the most blight resistant of all. Figure 3, from a photo taken in September, 1941, shows the size and growth habit of some of these trees, then fifteen years old, at our plantations at Hamden, Connecticut. The spreading habit is characteristic of the Chinese species, and the photograph shows how unsuited the tree is for forest planting. In closely planted stands, however, the individuals would probably be more erect. In 1935 we set out a large number of these trees, from nuts obtained from open pollination, to determine what will be their form in a close stand planted six feet apart. As a matter of fact, there seems to be considerable variation in this species as regards habit of growth. There are reports of fairly tall individuals with an excurrent growth habit. It would, of course, be desirable to use such stock for crossing: we should like to obtain scions of such stock.

Our Chinese-American hybrids, therefore, are still young, so that we are not yet prepared to make a preliminary statement about their genetic situation.

OTHER HYBRID FORMS.—Our past reports show a large number of crosses which do not seem to bear any definite relation to the problem in hand. These were made for various reasons, stated in the reports. If time permits, we shall try to develop them further. Most of them, however, relate to some quality of the nuts—fruitfulness, early ripening, size, flavor, number in a bur, or the everbearing character. However, the qualities of the nuts are economically of transitory importance as compared with the value of good chestnut timber.



Fig. 3. Row of Chinese chestnut (*Castanea mollissima*), fifteen years old, showing low-growing, deliquescent habit with thick, sturdy, lateral branches, often almost horizontal. Photo by Louis Buhle, September 13, 1941. (Photograph loaned by Brooklyn Botanic Garden.)

HYBRIDS OF 1941.—A total of 260 hybrid nuts was harvested in 1941. In line with what has been said above, 135 of these were obtained from backcrosses of Japanese-American hybrids, 50 came from interbreeding of Japanese-American hybrids, and 24 were the result of first crosses of the American chestnut (*C. dentata*) with our best Japanese trees. As usual (since 1934) all of the cross pollinating was done at our plantations at Hamden, Connecticut. We received pollen by mail from various sources, and a list of donors with our acknowledgment of their co-operation has been presented in the thirty-first Annual Report of the Brooklyn Botanic Garden (Graves, 1942).

In table 1 the female parent is given first. The combinations new to science are preceded by an asterisk.

VARIATION IN THE CHESTNUT.—In our last report we discussed the question of the occurrence of variation in the American chestnut, particularly along the line of ever increasing resistance to the parasitic fungus which induces the blight. The possibility of the occurrence of variation was based partly on the fact that at the present time vast numbers of young basal shoots are arising from stumps or the bases of diseased trunks all over the natural range of the American chestnut. This situation, as has been proved by the writer (1926), is due to the fact that the roots of chestnut resist the attacks of the fungus with much greater vigor than do the parts above ground. The condition has also been likened to a pruning operation in which the upper parts are cut off, the lower parts being thus forced to develop. The case of

TABLE 1. *Castanea* hybrids of 1941.

	No. of nuts obtained
<i>Castanea crenata</i> × <i>C. dentata</i>	24
<i>C. crenata</i> × <i>C. pumila</i>	10
<i>C. crenata</i> × (<i>C</i> × <i>D</i>) ^a	84
(<i>C</i> × <i>D</i>) × <i>C. crenata</i>	51
(<i>C</i> × <i>D</i>) × (<i>C</i> × <i>D</i>).....	50
*(<i>S8</i> ^b × <i>C. sativa</i>) × <i>C. crenata</i>	15
*(<i>S8</i> × <i>C. sativa</i>) × [(<i>C. mollissima</i> × <i>C. Se-</i> <i>guinii</i>) × <i>C. sativa</i>].....	1
*(<i>C</i> × <i>D</i>) × [(<i>C</i> × <i>D</i>) × (<i>C</i> × <i>D</i>)].....	16
<i>C. mollissima</i> × <i>C. dentata</i>	3
<i>C. mollissima</i> × (<i>C. dentata</i> × <i>C. mollissima</i>)	3
* <i>C. mollissima</i> × (<i>C. mollissima</i> × <i>C. dentata</i>)	3
Total	260

^a *C* × *D* = *C. crenata* × *C. dentata*.

^b *S8*, one of the Van Fleet chestnut hybrids, is apparently a combination of *C. crenata* and *C. pumila*. The *S8*'s in our plantation are from open pollinated seedlings of *S8*.

Cytisus Adami has been cited, the upper part of a branch of which was cut off by Beyerinck (1900), the lower part, being thus forced to grow out, showing segregation and appearing in quite a different form. This example, as has been pointed out by one of our correspondents, was unfortunate, since in all probability, *Cytisus Adami* is a graft hybrid (Jones, 1934), although De Vries (1910) did not think so.

The fact remains, however, that a very large number of these basal shoots are being developed, and probably will continue to be developed for many

years to come, affording plenty of opportunity for natural variation. The possibility of variation in the direction of disease resistance should not be ignored. We are, therefore, planting all nuts of *Castanea dentata* sent us from many parts of the eastern states. Last fall we received them from seven states. If nuts are sent, they should be wrapped in moist cotton or sphagnum and should be mailed as soon as possible after they are gathered. We have learned that drying out kills the embryo. Address: A. H. Graves, Brooklyn Botanic Garden, 1000 Washington Avenue, Brooklyn, New York.

SUMMARY

In the F_1 Japanese-American hybrid chestnuts the American parent is incompletely dominant. In order to continue breeding for the desired result, namely a blight-resistant chestnut of tall erect

growth, suitable for a timber tree, the F_1 hybrids are being kept in vigorous condition by a kind of bridge grafting. No definite statement of the genetic situation in the Chinese-American hybrids can yet be made.

A situation in the breeding of certain types of tobacco which is similar in some respects to that of the Japanese-American hybrids of chestnut is briefly described.

A record of the results of breeding of various chestnut species and hybrids in 1941 is given, including four combinations new to science.

It is stated that the possibility of natural variation in the chestnut in the direction of disease resistance should not be ignored, and in this connection a plea is made for native nuts to be sent to the Brooklyn Botanic Garden.

BROOKLYN BOTANIC GARDEN,
BROOKLYN, NEW YORK

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A CYTOLOGICAL INVESTIGATION OF POLYGONATUM USING THE COLCHICINE-POLLEN TUBE TECHNIQUE¹

O. J. Eigsti

POLYGONATUM, the Solomon's Seal, is represented in many localities in eastern and central North America. In spite of this wide distribution, the abundance of cytological material which each plant affords, and the simplicity of study by the aceto-carmin technique, not much information is recorded concerning the cytology of the American

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species of this genus (Cardiff, 1906; Cartledge, 1933). More is known about the Asiatic and the European species (Matsuura and Sutô, 1935; Sutô, 1936; Hasegawa, 1933; Tischler, 1927, 1937, 1938). Although three taxonomic treatments have been published since 1915 (Farwell, 1915; Gates, 1917; Bush, 1927) which were intended to solve the taxonomic difficulties encountered in classifying the species by using the available manuals, the differentiation of those species having glabrous leaves is still difficult, and the proper identification of them is a vexing task. It is true that most taxonomic keys are unsatisfactory for the genus; however, recent treatments (Farwell, 1915; Gates, 1917; Bush, 1927) have not clarified the situation entirely. Deam (1940), Wiegand and Eames (1925) and undoubtedly others have found the definition of a species rather vague and the limits of the species indefinite. An objective of this study is to show how cytologi-

cal information has a bearing upon the taxonomic situation, since the problem of species relationships is closely related to the occurrence of tetraploidy among species of the genus.

Incidental to a study of the effects of colchicine upon mitotic activities of the pollen tube of *P. commutatum* (R. & S.) Dietr. (sensu Gray's Manual, 7th ed.) the present writer discovered that this species is a tetraploid ($2n=40$) (Eigsti, 1940a, 1940b). Additional collections of plants from other regions revealed that there are diploid plants ($2n=20$) very much like the tetraploid plants ($2n=40$) except for size (Eigsti, 1940d, 1941a). However, certain diploids with foliar pubescence (*P. pubescens* (Willd.) Pursh, $n=10$) are taxonomically different from either of the glabrous species, *P. biflorum* (Walt.) Ell. and *P. canaliculatum* (Willd.) Pursh. As early as 1917 Gates (1917) called *P. giganteum* Dietr. a mutant, but apparently no cytological verification of this assumption was published. Asa Gray recognized the *gigas* features of the tetraploid (Eigsti, 1941b) by naming the large species the "Great Solomon's Seal" and the smaller one the "Small Solomon's Seal," a distinction often correlated with polyploidy.

The method of securing cytological information by the use of pollen-tubes and the colchicine technique represents a new approach for cytotaxonomic investigations. A haploid complement of chromosomes in the generative cell is used for the basic morphological studies of the chromosomes. The chromosomes are scattered in the pollen tube and in such position are counted easily and measured with greater accuracy because of the straightness of the metaphasic chromosomes (Eigsti, 1940c). Also the region of the kinetochore can be located in a precise fashion, thereby facilitating comparative studies of the arms of the various chromosomes of the complement.

MATERIALS AND METHODS.—Because numerous requests have been received in regard to this pollen tube technique, more than the usual detail of procedure is given in the following ten steps.

(1) Flowers at the time of anthesis are brought to the laboratory for inoculations.

(2) A solution of sucrose and agar is prepared by dissolving 2 grams of agar and the proper amount of sucrose in 100 cc. distilled water (pH 6.5–7.0). The amount of agar used is twice the concentration required for germination because a dilution is made before the final spreading of the film over the slide. Likewise, the concentration of sucrose is twice that required for each particular type of pollen for reasons made clear in step (4).

(3) A 0.02 per cent aqueous solution of colchicine is prepared.

(4) Colchicine solution (3) is mixed with the sucrose-agar mixture (2) in equal proportions. To effect a proper mixture, calibrated vials are used.

(5) A vial (about 6 cc. capacity) containing sucrose-agar-colchicine is kept in a small, wide-

mouth bottle which serves as a water bath (temperature about $48^{\circ}\text{C}.$).

(6) A thin film of colchicine-sucrose-agar, sufficient to cover a glass slide, is made with a camel's hair brush (no. 3).

(7) Immediately after the film is spread pollen is introduced onto the medium by shaking the flower over the slide or by dipping the anther into the substrate.

(8) After inoculation the slide is placed in a specially designed germinating chamber (Eigsti, 1940c). This chamber is made of galvanized iron and constructed to hold slides over a water solution.

(9) The slide is kept in the chamber for at least twelve hours and usually not more than twenty-four hours. After this interval the preparation is ready for fixation, staining, and permanent mounting in balsam.

(10) The fixation and staining procedures can be outlined in eight steps, *viz.*, (a) stain in Belling's aceto-carmin fifteen minutes; (b) rinse in 45 per cent glacial acetic acid followed by a rinse in glacial acetic; (c) $\frac{1}{2}$ glacial acetic- $\frac{1}{2}$ tertiary butyl alcohol, ten minutes; (d) tertiary butyl alcohol, fifteen minutes; (e) $\frac{1}{2}$ tertiary butyl- $\frac{1}{2}$ xylol, ten minutes; (f) pure xylol, ten minutes, repeat this once; (g) mount in balsam.

This method provides slides with scattered metaphasic chromosomes in the pollen tube. In the region of the kinetochore the daughter chromosomes remain closely associated. However, at other points along the chromosome the separation is more or less complete; hence the length of chromosomal arms can be determined for each chromosome. It was possible to measure images of chromosomes by projecting a negative of the photomicrograph. This gave a magnification of $\times 17,500$. By means of a calibrated scale the actual lengths of chromosomes are expressed in microns (fig. 39–46).

Meiotic studies were made from aceto-carmin preparations of pollen mother cells, and these are illustrated by camera lucida drawings (fig. 30–33). Stomatal diagrams (fig. 37–38) were made from the photomicrographs of leaf portions of lower epidermis mounted in aceto-carmin. The illustrations of pollen grains were taken from ungerminated grains on slides used in chromosomal studies (fig. 34–36).

A 2 mm. apochromatic oil immersion objective and an $8\times$ compensating ocular were used in making the photomicrographs. A $10\times$ compensating ocular was substituted for the $8\times$ ocular in making the camera lucida drawings. The source of light was a Bausch and Lomb filament lamp and the light was filtered by a Wratten B filter No. 58.

OBSERVATIONS.—*The occurrence of tetraploidy.*—Tetraploid complements of representatives of *Polygonatum* are shown by photomicrographs (fig. 1–2, 5–9, 23–27) and the diploid complements are represented by figures 3–5, 10–22, and 28. Polyploidy was discovered when duplicate sets of chromosomes among the haploid complement of the pollen tube of

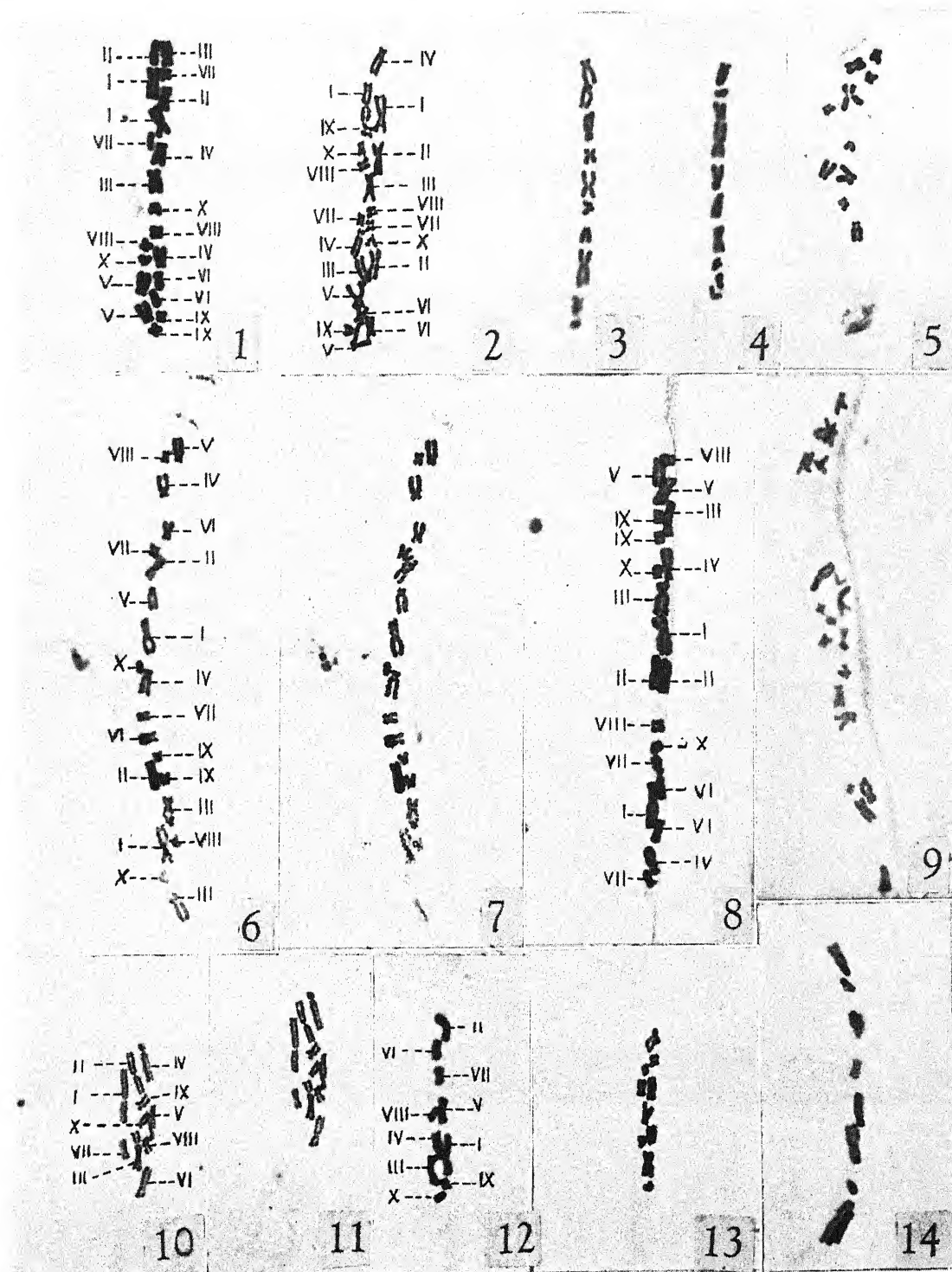


Fig. 1-14. Photomicrographs of chromosomes in pollen tubes of *P. canaliculatum* ($n=20$); *P. pubescens* ($n=10$); and *P. biflorum* ($n=10$); location of each collection given in table 2. All magnifications $\times 875$.—Fig. 1. Minnesota; Roman numerals indicate sizes and correspond to numerals elsewhere in figures where this collection is described; I-I are the largest pair while X-X are the smallest pair of homologous chromosomes; secondary association is shown by location of chromosomes with same numeral such as I, II, V, VI, VIII, IX, X.—Fig. 2. Oklahoma ($n=20$); 10 pairs of unseparated metaphasic chromosomes; secondary association of I, V, VI, VIII, IX.—Fig. 3. *P. pubescens*, Ohio, $n=10$.—Fig. 4. *P. pubescens*, Connecticut, $n=10$; two levels of focus.—Fig. 5. *P. biflorum*, Maryland, $n=10$; unusually

TABLE 1. *A list of chromosome numbers in the genus Polygonatum.*

Species	Chromosome number		Investigator and date
	n	2n	
<i>Polygonatum japonicum</i>	10	20	Hasegawa '33
<i>P. humile</i>	10	20	Hasegawa '33; Matsuura and Sutô '35
<i>P. lasianthum</i>	10	20	Hasegawa '33; Matsuura and Sutô '35
<i>P. silvicolum</i>	10	..	Hasegawa '33
<i>P. falcatum</i> (<i>P. latifolium</i> var. <i>commutatum</i> Bak.)	9	18	Hasegawa '33
<i>P. Maximowiczii</i>	10	..	Matsuura '35; Matsuura and Sutô '35
<i>P. hondoense</i>	11	..	Matsuura '35; Matsuura and Sutô '35
<i>P. biflorum</i>	7-8	..	Cardiff '06
<i>P. officinale</i> var. <i>japonica</i>	10	..	Hasegawa '33
<i>P. multiflorum</i>	9	..	v. Berg '33
<i>P. verticillatum</i>	14	..	v. Berg '33
<i>P. unknown species</i>	27	..	v. Berg '33
<i>P. latifolium</i>	9	..	v. Berg '33
<i>P. officinale</i>	10	..	v. Berg '33
<i>P. commutatum</i>	10	..	Cartledge '33
<i>P. biflorum</i>	10	..	Cartledge '33
<i>P. multiflorum</i>	12	..	von Boenicke '11
<i>P. commutatum</i>	20	..	Eigsti '40
<i>P. biflorum</i>	10	..	Eigsti '40
<i>P. pubescens</i>	10	..	Eigsti '40
<i>P. canaliculatum</i>	20	..	Eigsti '40

P. canaliculatum were noted (Eigsti, 1940b) and when it was learned that this number represented a multiple of ten, the predominating number reported for the genus. As a matter of fact, a haploid number of twenty is not found in the published lists of numbers for species of this genus, whereas nine reports of ten chromosomes are recorded for the genus (Tischler, 1927, 1937, 1938; Matsuura and Sutô, 1935; Sutô, 1936; Cardiff, 1906; Cartledge, 1933). That pairs of homologous chromosomes do occur in the haploid complement is quite obvious if one uses enlarged photographs of individual chromosomes and arranges them in a series according to size from the largest chromosome I and I' to the smallest chromosome X and X' (fig. 23-27). Such duplication of pairs of homologues is not shown in diploid species (fig. 15-22). The individual chromosomes in certain figures can be located by means of Roman numerals adjacent to the chromosomes of the tetraploid (fig. 1, 2, 5-7), and diploid (fig. 10, 12, 13).

Colchicine prevents the formation of a metaphase plate, thus resulting in the scattering of unseparated daughter-metaphase chromosomes (fig. 1-14). This influence of colchicine is described in greater detail in previous papers (Eigsti, 1940a, 1940b). Nebel and Ruttle (1938) refer to the peculiar chromosomal formation as "ski-like." These formations are demonstrated in figures 15, 21, and 24. When reference is made below to "pairs of chromosomes," consideration is given only to homologous pairs of chromo-

somes characteristic of tetraploid complements such as chromosome I and I' of figure 24. The "pair" does not refer to the two daughter chromosomes associated together (fig. 24 I).

Throughout this study which involved numerous different plants of the genus, only two different haploid chromosomal counts were noted, *viz.*, ten and twenty (fig. 15-27). On the other hand Cardiff (1906) reported seven and eight for one American species *P. biflorum*; and among the entire list of chromosomes for *Polygonatum* (table 1) six other variations from ten and twenty are reported. Perhaps less variation of chromosome counts is to be expected when the present method of counting chromosomes from the pollen tube treated with colchicine is used. A record of chromosomal counts is presented in table 1 and the complete record of work for this present cytological survey is given in table 2.

Chromosomal morphology.—A scattered arrangement of chromosomes in pollen tubes treated with colchicine (fig. 1-14) facilitates studies of chromosomal morphology. The relative sizes of the arms of each chromosome are studied by comparing individual chromosomes of enlarged photomicrographs (fig. 15-27) as well as through measurements of chromosomes drawn to scale, with the length of each arm of the longer chromosome designated in microns (fig. 39-46). Because colchicine treatment accentuates the locus of primary constriction, or the kinetochore, a more accurate estimate of the lengths of the arms

good distribution of chromosomes.—Fig. 6-7. *P. canaliculatum*, Indiana, $n=20$; two levels of focus; chromosomes indicated by Roman numerals I to X and their corresponding pairs.—Fig. 8. Virginia, $n=20$; chromosomes unusually long.—Fig. 9. *P. canaliculatum*, Michigan; $n=20$.—Fig. 10-11. *P. pubescens*, New Hampshire, $n=10$; Roman numerals mark individual chromosomes; two levels of focus.—Fig. 12. *P. biflorum*, New Jersey, $n=10$; individual chromosomes labeled by Roman numerals; I is largest chromosomes and X the smallest.—Fig. 13. *P. pubescens*, Michigan, $n=10$.—Fig. 14. *P. pubescens*, Tennessee, $n=10$.

TABLE 2. A summary of the collections used for the cytological studies.

Species	Culture, date	Locality	Collector	Text figure	Chromosome number (n)	Remarks
1. <i>P. biflorum</i>	PM 1941	Prince Georges Co., Md.	Dr. Ronald Bamford, College Park, Md.	5, 15	n-10	Glabrous, small narrow leaf
2. <i>P. biflorum</i>	PNJ 1941	Middlesex Co., N. J.	Dr. M. A. Chrysler, New Brunswick, N. J.	12, 18, 39	n-10	Glabrous, small narrow leaf
3. <i>P. biflorum</i>	PO 1941	Franklin Co., Ohio	Dr. Clyde Jones, Columbus, Ohio	Glabrous, narrow leaf
4. <i>P. canaliculatum</i>	PFV 1939, 1940, 1941	Floyd Co., Va.	Dr. J. T. Baldwin, Jr., Ann Arbor, Mich.	8, 23, 45	n-20	Glabrous, large plant, broad leaf
5. <i>P. canaliculatum</i>	PN 1939, 1940, 1941	Cleveland Co., Okla.	Dr. O. J. Eigsti, Norman, Okla.	2, 24, 29, 35, 37, 44	n-20	Glabrous, small plants
6. <i>P. canaliculatum</i>	P- 1940, 1941	Cleveland Co., Okla.	Dr. O. J. Eigsti, Norman, Okla.	n-20	Glabrous, broad large leaves
7. <i>P. canaliculatum</i>	MI 1941	Monona Co., Iowa	Dr. George Goodman, Ames, Iowa	n-20	Glabrous, large
8. <i>P. canaliculatum</i>	SUI 1941	Johnson Co., Iowa	Dr. W. A. Anderson, Iowa City, Iowa	n-20	Glabrous, large
9. <i>P. canaliculatum</i>	MO 1941	Cole Co., Mo.	Mrs. H. H. Leake, Norman, Okla.	n-20	Glabrous
10. <i>P. canaliculatum</i>	PX 1941	Hennepin Co., Minn.	Dr. C. O. Rosendahl, Minneapolis, Minn.	1, 25, 43	n-20	Glabrous, small, "biflorum" plant, narrow leaf
11. <i>P. canaliculatum</i>	PI 1941	Hennepin Co., Minn.	Dr. C. O. Rosendahl, Minneapolis, Minn.	n-20	Glabrous, large
12. <i>P. canaliculatum</i>	PA7 1941	Washtenaw Co., Mich.	Dr. J. T. Baldwin, Jr., Ann Arbor, Mich.	9, 27	n-20	Glabrous, large
13. <i>P. canaliculatum</i>	IND 1941	Elkhart Co., Ind.	Dr. O. J. Eigsti, Norman, Okla.	6-7, 26, 36, 46	n-20	Glabrous, large
14. <i>P. canaliculatum</i>	PS 1940	Durham Co., N. C.	Dr. L. E. Anderson, Durham, N. C.	n-20	Glabrous, large
15. <i>P. canaliculatum</i>	PB 1941	McLean Co., Ill.	Dr. Opal C. Hartline, Bloomington, Ill.	Glabrous, large
16. <i>P. canaliculatum</i>	PV 1941	Clarke Co., Va.	Mr. Erich Steiner, Boyce, Va.	Glabrous, large
17. <i>P. canaliculatum</i>	PC 1941	Tolland Co., Conn.	Dr. G. S. Torrey, Storrs, Conn.	26, 17, 40	n-10	Pubescent, small narrow leaf
18. <i>P. pubescens</i>	PT 1940	Davidson Co., Tenn.	Dr. A. J. Sharp, Nashville, Tenn.	Pubescent, small
19. <i>P. pubescens</i>	PT 1941	Davidson Co., Tenn.	Dr. A. J. Sharp, Nashville, Tenn.	14, 22, 34	n-10	Pubescent, small
20. <i>P. pubescens</i>	PO 1941	Franklin Co., Ohio	Dr. Clyde Jones, Columbus, Ohio	3, 21, 38	n-10	Pubescent, small narrow leaf
21. <i>P. pubescens</i>	PE 1941	Penobscot Co., Me.	Dr. E. C. Ogden, Orono, Me.	Pubescent, small
22. <i>P. pubescens</i>	PNH 1940	Strafford Co., N. H.	Dr. Albion R. Hodgdon, Durham, N. H.	10-11, 20, 28, 42	n-10	Pubescent, small
23. <i>P. pubescens</i>	P5 1941	Wabash Co., Minn.	Dr. C. O. Rosendahl, Minneapolis, Minn.	16	n-10	Pubescent, small
24. <i>P. pubescens</i>	P4 1940	Washtenaw Co., Mich.	Dr. J. T. Baldwin, Jr., Ann Arbor, Mich.	n-10	Pubescent, small
25. <i>P. pubescens</i>	P4 1941	Washtenaw Co., Mich.	Dr. J. T. Baldwin, Jr., Ann Arbor, Mich.	13, 19, 41	n-10	Pubescent, small

Summary:

Collections of plants from 25 localities:

P. canaliculatum (n-20) 13*P. pubescens* (n-10) 9*P. biflorum* (n-10) 3

—

25

and the morphology of the chromosome can be made using this reagent (fig. 15-27, 28, 29, 39-46).

Matsuura and Sutô (1935) list both primary and secondary constrictions among the complements of *P. humile* and *P. Maximowiczii*. They conclude for *P. humile* that there are seven chromosomes with sub-terminal, and three with sub-medial insertions, whereas the present study of complements from treated pollen tubes reveals six with sub-terminal insertions (fig. 28, III, IV, V, VI, IX, X), and four with sub-medial insertions (fig. 28, I, II, VII, VIII) among the species with a haploid complement of ten (fig. 15-22, 28); and twelve sub-terminal (fig. 29) and eight sub-medial insertions among those with a complement of twenty (fig. 23-27, 29).

In general there are six long chromosomes and four short ones in diploids (fig. 15-22, 28), and twelve long chromosomes and eight short ones in tetraploids (fig. 23-27, 29). Certain preparations show less tightly coiled chromosomes (fig. 10, 20, 24), and, because of this increased length, gradations of size are more easily recognized (fig. 20-24). A further classification of size is made by letters L, l, M, m, S, s, shown in figure 28; and L, L', M, M', m, m', S, S', s, s', are used to indicate homologous pairs of chromosomes in tetraploid plants (fig. 29). Individual chromosomes are indicated by Roman numerals (fig. 1, 2, 6, 8, 12, 10, 13, 14, 15-29, 39-46).

A study of the total length of individual chromosomes and the combined lengths of all chromosomes of a haploid complement is easier to make from the chromosomes in treated pollen tubes than from chromosomes found in the usual mitotic and meiotic stages. Of the four examples of diploid species measured (fig. 39-42) the average total length is 45.0 microns and the average for the four tetraploid species (fig. 43-46) is 83.8 microns. The latter figure is almost twice that of the former.

An idiogram of the tetraploid species was constructed from the average length of each arm of each chromosome (fig. 47) and the average length of each whole chromosome (fig. 47). A similar idiogram was made for the diploid species (fig. 48). The average total length of chromosomes of the five tetraploids measured is 81.1 microns (fig. 47) while the average total length of the chromosomes of seven diploids measured is 39.0 microns (fig. 48).

Of all the chromosomes measured in this study the longest was 11.5 microns (fig. 42 I), and the shortest 1.75 microns (fig. 41 X). Variability in size of individual chromosomes between different complements is noticeable, for instance, the chromosomes shown in figure 41 are much smaller than those represented in figure 42. A statistical analysis correlating the size of the plant with size of the chromosomes was not made; however, the largest tetraploid plant collected (table 2, no. 13) had the largest chromosomes (fig. 45), whereas the smallest tetraploid plant (table 2, no. 10) had the smallest total length of chromosomes (fig. 43). Darlington (1932) discusses the relation of the length of chromosomes to genotypic control. Recently Swanson (1942) re-

ported statistically significant size differences of chromosomes among different clones of plants belonging to the same genus. Further correlations between chromosomal size and characteristics of plants can be subjected to more intensive study with the present method of securing preparations.

Duplication of homologous chromosomes.—The chromosomal pairs of the tetraploid races often appear close together as shown by pairs I, II, V, VI, VIII, IX, and X in figure 1. A similar situation is found in figure 2 and to a limited extent this is true for chromosomes shown in figures 6 and 8. This is a case of secondary association of chromosomes. Since the cytoplasm is constantly moving in the pollen tube, there is opportunity for considerable rearrangement; in spite of this movement, the homologous chromosomes are often found close to each other.

Twenty distinct bivalents did not appear in the aceto-carmin preparations of meiosis in *P. canaliculatum* as found near Norman, Oklahoma (fig. 30-33). There is rather a grouping, or multivalent association (fig. 30-33). Cardiff (1906) noted difficulties in distinguishing the line of synapsis of the chromosomes in his studies of meiotic metaphases. Matsuura and Sutô (1935) reported ten bivalents for *P. Maximowiczii*, an Asiatic species; however, they mention that the "individual chromosomes are hardly discernible." The multivalent association (fig. 30-33) is indicative of homologies between the chromosomes of the duplicate sets, and further homology is brought out by a comparison of the pairs of enlarged chromosomes (fig. 23-27, chromosomes I-I', II-II', III-III', IV-IV', V-V', VI-VI', VII-VII', VIII-VIII', IX-IX', X-X').

Tetraploidy and the gigas complex.—The *gigas* characteristics associated with the tetraploid condition are quite obvious among those species with $2n=40$ chromosomes. As a rule the plants are more robust, the leaves are broader and thicker, the plants are greener, the rhizomes are more massive, the epidermal cells, especially the guard cells, are larger (compare fig. 37, a tetraploid, with fig. 38, a diploid), and the pollen grains of diploids (fig. 34) are smaller than the pollen grains of tetraploids (fig. 35-36). These are the usual quantitative morphological characteristics which differentiate diploid plants from natural and induced tetraploids (Müntzing, 1936). Müntzing (1932) states that "a characteristic feature of auto-polyploids is their vegetative vigor which is often striking enough to be called gigantism." *P. canaliculatum* ($2n=40$) is an excellent example of a species showing such *gigas* characters. However, tetraploid plants which are small and otherwise typical *biflorum* (table 2, no. 10) occasionally appear in the collections. While many characteristics point toward an autopolyploid condition, the present writer cannot conclusively prove this to be the case with the limited collection of plants and facts available. Systematists have recognized the morphological differences here found, as shown by their descriptions in the keys and manuals. Gates

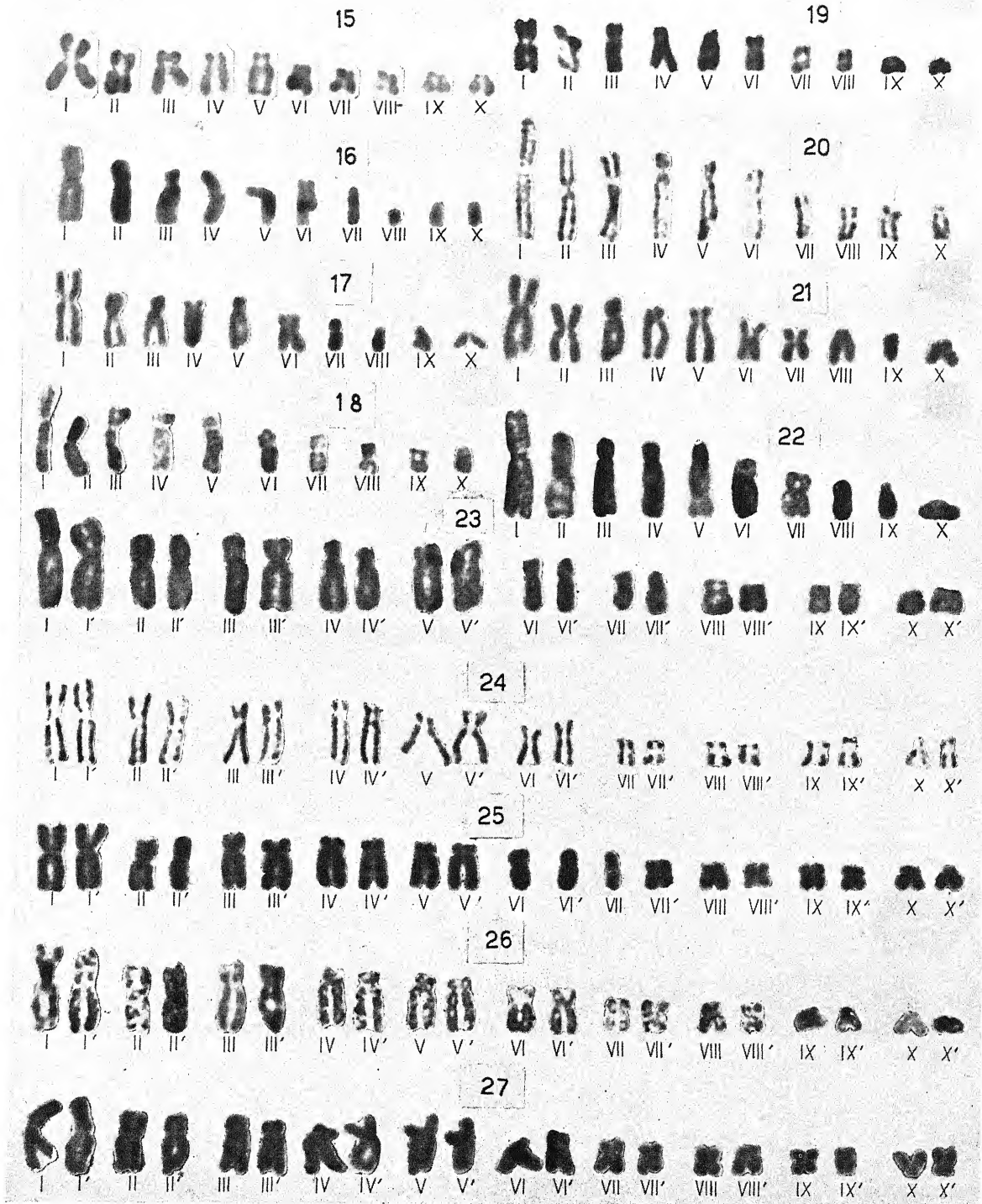


Fig. 15-27. Enlargements of photomicrographs arranged in relation to size of chromosomes; largest chromosome on the left marked I in species with haploid number of ten and I-I' in species with $n=20$ chromosomes; certain collections shown in figures 1-14 represented; primary constriction appears unstained and in many cases marks the point where daughter chromosomes remain attached; differences in widths and length related to spiralization. All magnifications $\times 1,650$.—Fig. 15. *P. biflorum*, Maryland (fig. 5), twenty unseparated daughter chromosomes; four sub-medial chromosomes I, II, VII, VIII; six sub-terminal chromosomes, III, IV, V, VI, IX.—Fig. 16. *P. pubescens*, Minnesota;

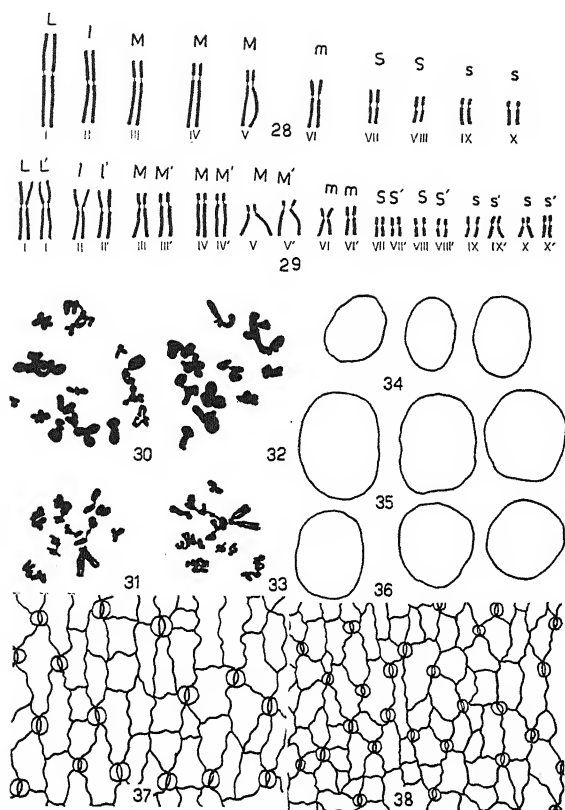


Fig. 28-38.—Fig. 28. Diagrammatic reconstruction of the chromosomal complement of *P. pubescens*, New Hampshire (fig. 10-11, 20); part of construction taken from photomicrograph and part from camera lucida drawing of the figure as it appears on the preparation; individual chromosomes are numbered by Roman numerals as in other figures; a more detail size classification is made by letters; L—largest, sub-medial insertion of the chromosome, l—next largest, sub-medial insertion chromosome; M—three medium sized chromosomes with sub-terminal insertions; m—next to medium size chromosomes with sub-terminal insertions; S—two small chromosomes sub-medial; s—two smallest chromosomes sub-terminal. $\times 750$.—Fig. 29. *P. canaliculatum*, Oklahoma (fig. 2, 24); reconstructed as figure 28; except for size of chromosomes of entire complement the general characteristics of chromosomes I-I', and others to X-X' are very similar to those in figure 28; size of chromosome by letter indicated through letters L-L', l-l', M-M', m-m', S-S', s-s'. $\times 750$.—Fig. 30-33.

chromosomes not distinct for arm length.—Fig. 17. *P. pubescens*, Connecticut (fig. 4); two levels of focus.—Fig. 18. *P. biflorum*, New Jersey (fig. 12); terminal attachment of X shown.—Fig. 19. *P. pubescens*, Michigan (fig. 13); small chromosomes; four sub-medial; six sub-terminal.—Fig. 20. *P. pubescens*, New Hampshire (fig. 10-11); loose spirals characteristic of this view; four sub-medial insertions I, II, VII, VIII; six sub-terminal insertions III, IV, V, VI, IX, X; six long chromosomes I-VI; four short chromosomes VI-X.—Fig. 21. *P. Pubescens*, Ohio (fig. 3); longest chromosome I appears much longer than the next largest size l.—Fig. 22. *P. pubescens*, Tennessee (fig. 14); six long chromosomes and four short.—Fig. 23. *P. canaliculatum*, Virginia (fig. 8); duplication of pairs of chromosomes marked by morphological features of the chromosomes; I-I' largest pair; V-V' particularly similar; also VI-VI', II-II'; twelve long chromosomes and eight short chromosomes.—Fig. 24. *P. canaliculatum*, Oklahoma (fig. 2); location of individual chromosomes in pollen tube figure listed in figure 2; similar morphology of pairs is shown especially in chromosomes I-I', IV-IV', V-V'; twelve long chromosomes and eight short chromosomes.—Fig. 25. *P. canaliculatum*, Minnesota (fig. 1); duplication of chromosomes in haploid complement shown by chromosomes I-I', II-II', III-III', IV-IV', V-V', VI-VI', X-X'; twelve long chromosomes and eight short ones; chromosome VII turned on edge.—Fig. 26. *P. canaliculatum*, Indiana (fig. 6-7); duplication shown by pairs of chromosomes.—Fig. 27. *P. canaliculatum*, Michigan (fig. 9); pairs of chromosomes in duplicate.

(1917) undoubtedly had a duplication of chromosomes in mind when he characterized *P. giganteum* as a mutant, and it must be recognized that several other plants in his classification were probably tetraploid. However, no cytological confirmation was reported in the literature.

DISCUSSION.—Speciation and polyploidy.—Because quantitative morphological characters are closely related to the problem of speciation, and because these are in turn associated with chromosomal differences taxonomists need all the cytological information possible before they can advance very far in their knowledge of species differentiation in this genus (Darlington, 1932). Cytological and genetical investigations of numerous workers have added much to our knowledge of species-formation in plants (Müntzing, 1932—*Galeopsis Tetrahit*; Huskins, 1930—*Spartina Townsendii*; Manton, 1935—*Nasturtium officinale*; Babcock, 1942—*Crepis*; and others). As a consequence of such cyto-genetic studies, the rôle of polyploidy in evolution is probably better understood than that of any other kind of hereditary change (Stebbins, 1940). Particularly helpful is the cytological information where systematists are confronted with closely intergrading forms which can be distinguished only by chromosomal differences. Often the sources of greatest vexation to the systematist provide the "keystone for evolutionary theory" (Dobzhansky, 1941). Speciation has occurred in *Polygonatum* through duplication of chromosomes. Accompanying this duplication, however, there are but few distinguishing qualitative characteristics which separate the diploid and tetraploid plants. Consequently it is difficult to make a satisfactory separation of species on the basis of taxonomic characters.

Cyto-taxonomy.—When polyploidy was discovered in the genus (Eigsti, 1940b) the bearing of this discovery upon taxonomic problems was obvious. Over two decades ago, Farwell (1915) pointed out

P. canaliculatum, Oklahoma; four views of first meiotic metaphase; in no figure are twenty bivalents distinguishable; homologies of chromosomes demonstrated by the association. $\times 875$.—Fig. 34. *P. pubescens*; diploid pollen grains; camera lucida drawing. $\times 245$.—Fig. 35-36. *P. canaliculatum*; tetraploid pollen grains; $\times 245$.—Fig. 37. *P. canaliculatum*; lower epidermis; $\times 100$.—Fig. 38. *P. pubescens*; lower epidermis; $\times 100$.

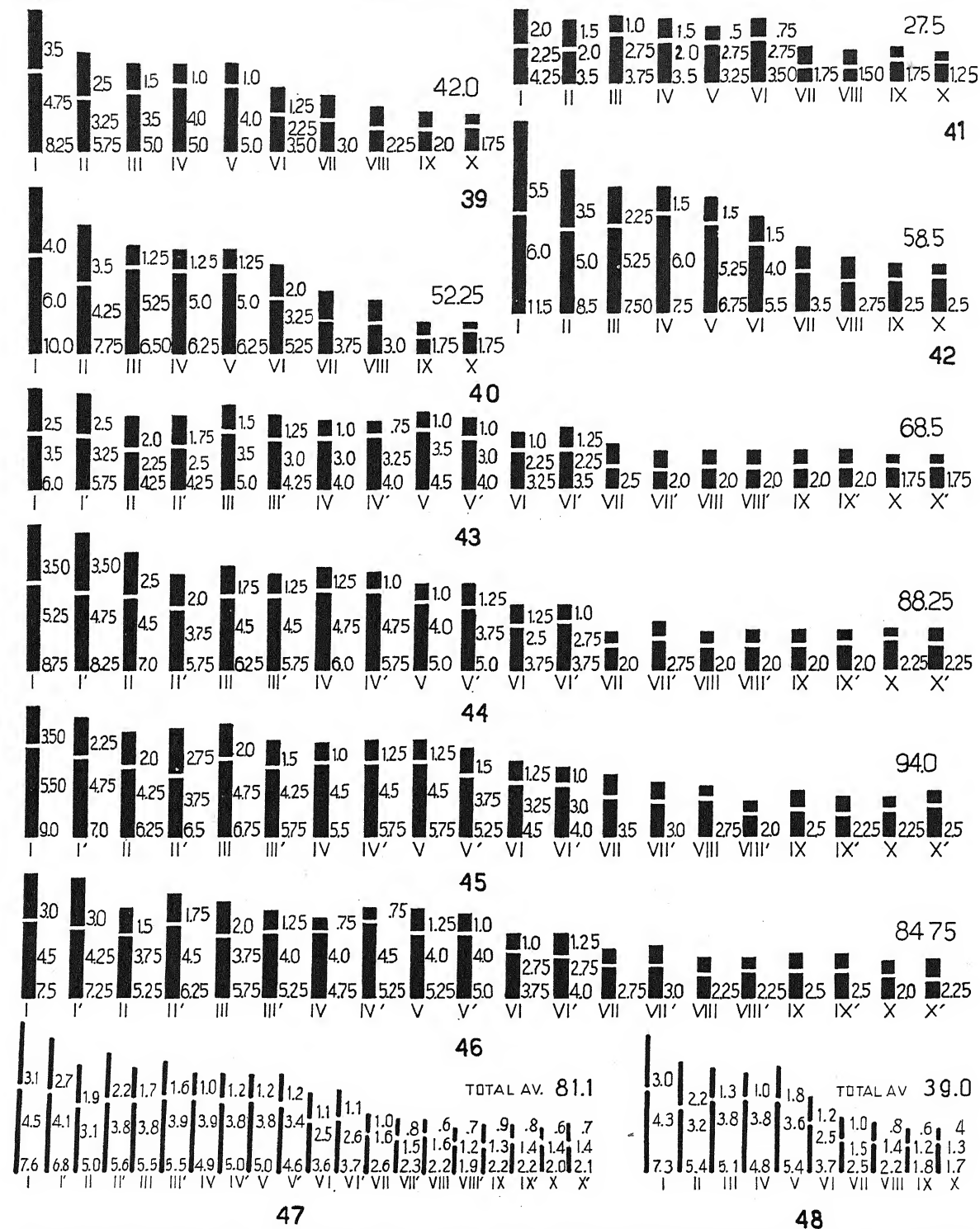


Fig. 39-48. Diagrammatic constructions from measurements of projected photomicrograph negatives magnified $\times 17,500$. The length of each arm of the chromosome listed for chromosomes I to VI; only total length listed for chromosomes VII to X; numbers at the right of each chromosome are as follows: top number is the short arm length; middle number lower arm; bottom number total length of the chromosome. Measurements from screen were made by a ruler calibrated to microns. Each micron equals 10 mm.; the white space marks location of kinetochore which separates the two arms of the chromosomes; total length of all chromosomes of a complement is listed above chromosome IX and X for each set.—Fig. 39. *P. biflorum*, New Jersey (fig. 18, 12).—Fig. 40. *P. pubescens*, Tennessee (fig. 22, 14); six long chromosomes, 5.25 microns or longer, four short chromosomes, 3.75 microns or shorter; total length 52.25 microns.—Fig. 41. *P. pubes-*

difficulties in classification of species in his monograph of the genus. Later Gates (1917) described the situation in this genus as "chaotic." Bush (1927) placed the problem among the unsolved ones in his study of the flora of Missouri and Deam (1940) records his observations and difficulties in dealing with the genus in Indiana.

Certain taxonomists have grouped all North American forms under *P. multiflorum* and others have admitted as many as twelve species (Farwell, 1915). Speciation from the cyto-taxonomic point of view, as shown by studies of plants from a number of localities (table 2), is best outlined under two distinct complexes separated by the presence or absence of foliar pubescence. The pubescent complex embraces the species *P. pubescens*, a diploid with $2n=20$ (fig. 3-4, 10-11, 17, 19, 20-22, 40-42). The glabrous complex includes two cytological types, *P. biflorum*, a diploid species with $2n=20$ (fig. 5, 13, 15, 18, 39), and *P. canaliculatum* with $2n=40$ (fig. 1-2, 5-9, 23-27). *P. canaliculatum* is a large plant with broad leaves clasping at the base and the *gigas* complex of characters, whereas the smaller species, *P. biflorum*, has narrow leaves, a smaller stature, and in general is a less sturdy looking plant. In the one manual used widely for this region (Gray's 7th ed.), only the diploid and tetraploid species are admitted, as *P. biflorum* and *P. commutatum*.² The third species, with foliar pubescence, found in many localities is omitted. Had the names *P. pubescens* and *P. canaliculatum* of the first edition been retained, much confusion could have been avoided.³ According to a study by Farwell (1928) there is a fourth species, *P. melleum*, with flowers honey yellow and distinct from the three species mentioned here. In the present cytological survey this species was unfortunately never procured. Future cytological analysis of this species is anticipated.

Distribution of species of Polygonatum.—Among the twenty-five collections (table 2) thirteen were classified as *P. canaliculatum*, nine as *P. pubescens*, and three as *P. biflorum*. Of the thirteen collections of *P. canaliculatum* eleven were analyzed cytologically as tetraploids, $2n=40$ (fig. 23-27, 43-46); of the nine *P. pubescens*, seven were diploids, $2n=20$ (fig. 16, 17, 19, 20, 21, 22, 40-42); and of the three *P. biflorum*, two were diploids, $2n=20$ (fig. 15, 16, 39). The tetraploids have a wider distribution and seem to be more abundant than the diploid plants. This observation is in harmony with previous

² Dr. W. A. Anderson, University of Iowa, Iowa City, Iowa, informs me in a personal communication that *P. canaliculatum* is a form of the plant which has been called *P. commutatum*.

cens, Michigan (fig. 19, 13); chromosomes short throughout complement, total 27.5 microns.—Fig. 42. *P. pubescens*, New Hampshire (fig. 10-11, 20); unusually long chromosomes, six long chromosomes and four short ones.—Fig. 42. *P. canaliculatum*, Minnesota (fig. 1, 24); short chromosomes throughout the complement; twelve long chromosomes differentiated from eight short.—Fig. 44. *P. canaliculatum*, Oklahoma (fig. 2, 25); twelve long chromosomes and eight short ones.—Fig. 45. *P. canaliculatum*, Virginia (fig. 8, 23); longest chromosomes measured; total 94.00 microns.—Fig. 46. *P. canaliculatum*, Indiana (fig. 6-7, 26); two classes of chromosomes, twelve long and eight short chromosomes.—Fig. 47. Idiogram of tetraploid constructed from the averages of the arms of chromosomes of five different complements.—Fig. 48. Idiogram of diploid involving seven measurements of different complements.

studies of geographic distribution of tetraploid plants (Müntzing, 1936; Stebbins, 1940).

Wiegand and Eames (1925) have made a study of *Polygonatum* as it occurs in the region of the Cayuga Lake Basin in northwestern New York. In this flora two forms of the glabrous complex occur, viz., one with elliptic, lanceolate leaves (probably a diploid), and the other with broad leaves and coarse, erect stems (tetraploid), found in rich soils. From their description it is possible that these plants are examples of cytologically different forms which are not easily distinguishable taxonomically. In Indiana (Deam, 1940) the tetraploid (table 2, no. 13, fig. 26, 36, 46) is found along roadsides, and what is probably the diploid (*P. biflorum*) is from wooded areas. These differences in habitat are probably the result of the different physiological characteristics of diploids and tetraploids.

The seeds of all species are disseminated by birds, since the fleshy fruit is attractive to and eaten by them (Deam, 1940). Once a seedling is established in a new territory, the one with most vigorous vegetative propagation survives under the more adverse conditions and thus in a greater variety of situations. In this case the tetraploid, *P. canaliculatum*, is able to survive because these plants are robust and hardy and more easily propagated vegetatively. Deam⁴ finds that when the diploids and the tetraploids are established in a garden, the latter is more difficult to eradicate. Perhaps another factor to be considered is that tetraploids frequently exhibit greater variation in vigor than the corresponding diploids. Stebbins (1942) observed a greater variation in vigor among tetraploid progeny of *Stipa lepida* Hitchc. than among diploid. It seems plausible that the wider distribution and greater abundance of *P. canaliculatum* is probably due to these factors.

The diploid species is in flower earlier than the tetraploid species. In the garden at the University of Oklahoma diploid species flower about three weeks earlier than the tetraploids growing under similar conditions. Such differences in relation to physiological changes accompanying polyploidy are discussed by Stebbins (1940). A difference in period of flowering becomes a factor of great significance as an isolation mechanism between the diploid and tetraploid races. Furthermore, the different ecological relationships tend to isolate the species in different environmental areas.

SUMMARY

A cytological investigation of colchicine-treated pollen tubes of *Polygonatum* has been made.

³ This information was kindly contributed in a letter from Dr. W. A. Anderson.

⁴ Personal communication.

Duplication of sets of chromosomes in tetraploid plants was confirmed by comparison of the sizes of the various chromosomes of the complement and by the occurrence of a multiple series of chromosome numbers.

Colchicine facilitates the studies of chromosomal morphology because the chromosomes are straight and the region of the kinetochore is quite distinct. The scattered chromosomes of treated pollen tubes are easier to study as individual units.

In diploid species there are six sub-terminal and four sub-medial chromosomes; in tetraploids there are twelve and eight respectively. In general there are six long chromosomes and four short ones in diploids. In tetraploids there are twelve long and eight short chromosomes. Further size classification is made, using the letters L, l, M, m, S, and s. Individual chromosomes are indicated by Roman numerals.

The average total length of the chromosomes of seven complements among diploid species was 39.0 microns; the average of five tetraploid complements was 81.1 microns. The longest chromosome measured was 11.5 microns and the shortest 1.25 microns.

Secondary association was apparent in tetraploid complements. Further evidence of homologies among chromosomes of the tetraploid was secured from the

multivalent associations in meiotic studies in the Oklahoma collections.

The so-called *gigas* characters were found in *P. canaliculatum*, the tetraploid species. These characters which differentiate the diploid species from the tetraploid are quantitative rather than qualitative. Speciation has occurred by duplication of chromosomes in the genus *Polygonatum*. The polyploid series was found in the glabrous complex of this genus. *P. biflorum* is the diploid ($n=10$) and *P. canaliculatum* ($n=20$) is the tetraploid; both were found to be glabrous plants.

Tetraploids are more widely distributed than diploids. Increased hardiness, greater vigor and tendency toward more vegetative propagation in tetraploids are apparently factors responsible for the distributional differences between diploid and tetraploid species.

When both species are found in the same region, the diploid plants flower about one month earlier than the tetraploid plants, which is considered to be an important factor in the isolation of species of this genus.

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AUTOPOLYPLOIDY AND GEOGRAPHICAL DISTRIBUTION IN *CUTHBERTIA GRAMINEA* SMALL¹

Norman H. Giles, Jr.

THE RELATIVE rôles which various kinds of genetic change have played in the evolution of species continue to be of great interest to the cytogeneticist (Dobzhansky, 1941) and are increasingly being recognized as of fundamental concern to the systematist (cf. Huxley, *et al.*, 1940). Allopolyploidy is generally recognized as having been of exceptional importance as an evolutionary mechanism in the higher plants. The question of the relative significance of autopolyploidy, however, is not as well settled (Stebbins, 1940), although Müntzing (1936) and others have presented evidence indicating that it is probably of considerable consequence. The purpose of the present paper is to report some of the results of a study of intraspecific autopolyploidy in *Cuthbertia graminea*. The evidence in this case indicates that autopolyploidy has profound morphological and physiological effects on the individuals in which it occurs, and further, that it has resulted in striking changes in the composition of the natural populations of this species complex. It seems clear that the advantages resulting from autopolyploidy have been so considerable that autotetraploids now constitute a very large fraction, numerically and geographically, of the individuals in this taxonomic species.

THE TAXONOMY AND CYTOLOGY OF CUTHBERTIA GRAMINEA.—The genus *Cuthbertia* was erected by Small (1903) to include a homogeneous group of species in the Commelinaceae, which, though closely related to the well-known spiderworts of the *virginiana* complex of *Tradescantia* L., are clearly dis-

tinct. The genus as now recognized (Small, 1933) includes the three species *C. graminea*, *C. rosea* (Vent.) Small, and *C. ornata* Small. *Cuthbertia* is separable from *Tradescantia* on morphological, geographical, and cytogenetical grounds. Such differences have been pointed out by Anderson and Woodson (1935) and Anderson and Sax (1936), even though the former authors retain the species of *Cuthbertia* as varieties of *T. rosea* Vent. in their taxonomic monograph of species of *Tradescantia* occurring within the United States.

The only previous description of the chromosome complement for a species of *Cuthbertia* is that given by Anderson and Sax (1936), who found the somatic number of *graminea* to be twenty-four—eight chromosomes with approximately median, and sixteen with sub-terminal centromeres. The meiotic behavior was described as regular and the amount of visibly normal pollen as high. It has since become evident from the present studies that the plant which they examined must have been an autotetraploid, and that diploids and autohexaploids also exist in *graminea*. An examination of several specimens of *rosea* has so far revealed only diploids.

The types of chromosomes composing the basic complement of *C. graminea* are particularly clear at mitosis in the pollen grain (fig. 1 and 2). It is evident that four of the chromosomes have subterminal centromeres and two approximately median ones. Studies of chromosome pairing at meiosis in the three cytological forms which have been found to occur in this species have given the following results: in the diploids, six bivalents are regularly present at metaphase; in the tetraploids, bivalents and quadrivalents are the rule; while in the hexaploids, bivalents, quadrivalents, and hexavalents occur. In the somatic cells of the tetraploid, each of the chromosomes of the basic set is seen to be represented four

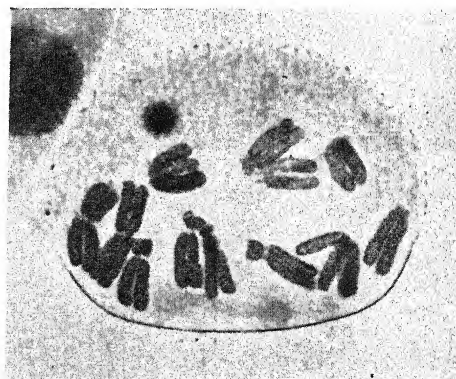
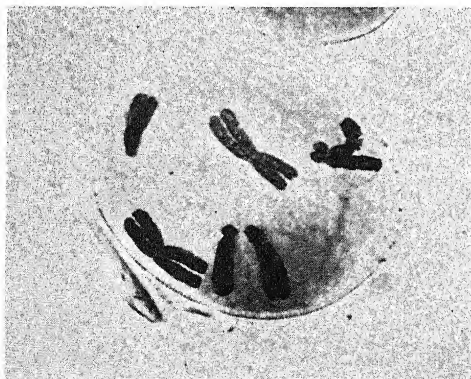


Fig. 1-2.—Fig. 1 (left). Haploid set of chromosomes of *Cuthbertia graminea* (2x) at metaphase of first post-meiotic (microspore) mitosis. $\times 1,000$.—Fig. 2 (right). Diploid set of chromosomes of *C. graminea* at the microspore division of an autotetraploid (4x). $\times 1,000$.

times, in a hexaploid each is represented six times. These observations provide cytological evidence for autopolyploidy. Further evidence is supplied by the fact that the polyploids are quite comparable morphologically to the diploids, except that they possess the "gigas" characteristics often associated with autopolyploidy (Müntzing, 1936). Also the diploids themselves are fully fertile and show no evidence of hybrid ancestry, as would be expected were the derived forms allo- rather than autopolyploids. A detailed cytological analysis, particularly of meiotic behavior in the three forms, will be deferred until a later paper.

It is clear that there are considerable differences between the chromosome complements of the species of *Cuthbertia* and those of the species of *Tradescantia* in the *virginiana* complex, since the latter have chromosomes all of whose centromeres are approximately median. These differences, together with the evidence from morphology and geographical distribution, imply a considerable genetic divergence of the two groups. It is not surprising under the circumstances, then, that extensive attempts which have been made during this study to make hybridizations between various species of the two genera have been unsuccessful.

In geographical distribution, the species of *Cuthbertia* are found in the South Atlantic Coastal Plain region (and rarely in the adjacent Piedmont province), from southeastern Virginia (Fernald, unpubl.) to southern Florida (Anderson and Woodson, 1935). Only *graminea* is found throughout this entire area, *rosea* occurring in the region north of Florida, and *ornata* only within the limits of that state.

COMPARISON OF THE DIPLOID AND POLYPLOID FORMS.—It has been pointed out by Anderson and Sax (1936) that the diploid and autotetraploid forms which occur in several species of the *virginiana* group of *Tradescantia* usually cannot be distinguished by gross size differences, although some general differences can be detected when the two forms have been identified cytologically. In the present case, however, there are considerable size differences between the diploids and the tetraploids of *C. graminea*, the latter being distinctly larger on the average. These differences are brought out in table 1 which records measurements of individuals whose chromosome numbers were known. For measure-

ments of leaf length and width, the maximum values found on a given plant were used to bring out the fact that on the average the organs of the tetraploids attain a considerably greater size than those of the diploids. The values for corolla diameter and sepal length are averages of several flowers from the number of plants indicated. In all the types of measurements for which standard errors have been calculated the differences between the means for the two types are highly significant. It is clear that diploids and autotetraploids vary in size of entire plants and plant organs about quite distinct means. Other measurements have indicated differences in stomatal size and number per unit area of leaf, and in pollen grain size, as would be expected. Studies in the field have shown that the tetraploids are in general much more vigorous plants than the diploids. The size of the tetraploid plants is considerably larger, and there are usually several stems per plant, whereas in the diploids there are usually only one or a few stems. Another indication of the greater vigor of the tetraploids is derived from comparisons of the two types growing under comparable greenhouse conditions. Here it has been found that the diploids show a much higher mortality than the tetraploids. The tetraploids flower more profusely than the diploids, and both types set seed readily in the field in most cases, even though preliminary studies in the greenhouse indicate that self-sterility is usually present, as is the case in the species related to *T. virginiana*. Propagation appears to be largely by seed rather than by vegetative means. There is some evidence from field observations that in the same latitude the diploids flower earlier than the tetraploids and probably have a shorter blooming season. Figure 3 shows a comparison of a diploid and an autotetraploid. These two plants were transplanted from the field to the greenhouse and are quite typical of the size differences found under natural conditions. In this connection, it is interesting to note that this size difference has led to some confusion among taxonomists, who have upon occasion identified the tetraploids of *graminea* as *rosea* because of their wider leaves. To one who is familiar with both species, however, the differences between even a tetraploid *graminea* and a diploid *rosea* are obvious at a glance.

It has not been possible to secure enough measurements on the hexaploids to permit adequate comparisons, since only two plants of this type were

TABLE 1. Comparative measurements of diploids and tetraploids of *Cuthbertia graminea*.

Character	Tetraploids			Diploids		
	Mean (mm.)	S. E.	No. plants measured	Mean (mm.)	S. E.	No. plants measured
Height of plant	205.0 \pm 4.4		53	158.0 \pm 4.6		18
Length of longest leaf	144.0 \pm 3.2		53	117.0 \pm 3.2		18
Width of widest leaf	4.1 \pm 0.1		53	2.2 \pm 0.1		18
Corolla diameter (average)	20.0		20	16.8		6
Sepal length (average)	5.3		20	4.3		6
Seed length	1.95 \pm 0.03		50 (seeds)	1.45 \pm 0.02		50 (seeds)
Seed weight (average)	1.48 mg.		162 (seeds)	0.73 mg.		176 (seeds)

TABLE 2. Somatic chromosome numbers found in *Cuthbertia graminea*.

	2n	Counts obtained at meiosis or in root tips				Plants collected	
		2n+1	Per cent	2n+2	Per cent	No. of localities	No. of plants
Diploids (2n=12)	13	0	0.0	0	0.0	12	28
Tetraploids (2n=24)	73	2	2.7	0	0.0	47	134
Hexaploids (2n=36)	1	0	0.0	1	50.0	2	2

available. However, it is clear from general observations that the hexaploids also show gigas characteristics, and in size of certain plant organs are larger than the tetraploids. They also appear to develop more slowly under greenhouse conditions. It is of interest to note that the first hexaploid found was specifically selected from among a population of tetraploids in the field because of the distinctly larger size of its buds and flowers.

SOMATIC CHROMOSOME NUMBERS.—In studying the cytology of three forms, an effort was made to obtain data on the prevalence of aneuploidy under natural conditions. Many of the preliminary counts made to determine the presence of polyploidy in plants collected in the field were based on studies at the microspore division, but these observations cannot be used with certainty to indicate the presence or absence of aneuploid types. Consequently, in as many plants as possible counts were obtained at meiosis; in a few, root-tip mitoses were used. The results are summarized in table 2. In the diploids, although the numbers are small, there were no cases of $2n+1$ types. It should be noted as well that, for plants in which counts were obtained at the microspore division only, there was no evidence of an excess of pollen grains with $n+1$ chromosomes. Probably all the twenty-eight plants had the basic number of twelve. In the tetraploids, however, of the seventy-five plants for which definite counts were obtained, two (2.7 per cent) were $4x+1$. In both exceptional plants one of the shorter chromosomes having a subterminal centromere was present as the

extra member of the complement. Only two hexaploids were obtained from collections in the field, but one of these had two extra chromosomes (both with subterminal centromeres) present.

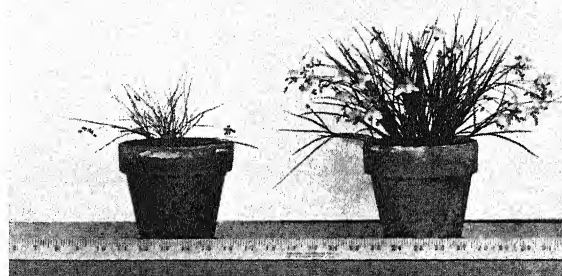


Fig. 3. Comparison of typical diploid (left) and auto-tetraploid plants of *C. graminea*.

ECOLOGICAL DISTRIBUTION.—The three species of *Cuthbertia* differ in their ecological distribution. The writer is familiar from field experience with only *C. graminea* and *C. rosea*, particularly the former. *Cuthbertia rosea* occurs in semi-mesic areas, such as oak woods, having a soil containing appreciable quantities of humus. On the other hand, *graminea* is

TABLE 3. Species observed in three typical areas where *Cuthbertia graminea* was collected for cytological examination. See text for discussion.

Area 1 (Spout Springs, N. C.)	Area 2 (Bryan County, Ga.)	Area 3 (Aiken, S. C.)
<i>Aristida stricta</i> Michx.	<i>Aristida stricta</i>	<i>Aristida stricta</i>
<i>Quercus laevis</i> Walt.	<i>Quercus laevis</i>	<i>Quercus laevis</i>
<i>Pinus australis</i> Michx. f.	<i>Pinus australis</i>	<i>Pinus australis</i>
<i>Cladonia sylvatica</i> (L.) Hoffm.	<i>Cladonia sylvatica</i>	<i>Nolina georgiana</i> Michx.
<i>Selaginella acanthonota</i> Underw.	<i>Selaginella ananthonota</i>	<i>Quercus Margaretta</i> Ashe.
<i>Sabulina caroliniana</i> (Walt.) Small	<i>Triplasis americana</i> Beauv.	<i>Quercus marilandica</i> Muench.
<i>Baptisia villosa</i> Ell.	<i>Stenophyllus Warei</i> (Torr.) Britton	<i>Sabulina caroliniana</i>
<i>Stillingia sylvatica</i> L.	<i>Serenoa repens</i> (Bartr.) Small	<i>Baptisia perfoliata</i> (L.) R. Br.
<i>Bivonea stimulosus</i> (Michx.) Raf.	<i>Eriogonum tomentosum</i> Michx.	<i>Bivonea stimulosus</i>
<i>Lasiococcus dumosa</i> (Andr.) Small	<i>Siphonochia</i> sp.	<i>Asclepias humistrata</i> Walt.
	<i>Sabulina caroliniana</i>	<i>Aureolaria pectinata</i> (Nutt.) Pennell
	<i>Bivonea stimulosus</i>	
	<i>Opuntia</i> sp.	
	<i>Dicerandra odoratissima</i> Harper	
	<i>Lacinaria</i> sp.	

typically a plant of definitely xeric areas of almost pure white sand. According to the description of ranges in Small (1933) and Anderson and Woodson (1935), *ornata* is found in the Florida "scrub," which is apparently a fairly distinct and characteristic plant association.

The localized ecological habitat to which *graminea* is largely restricted is commonly termed the "sandhills" whether it occurs in the more typical region of the Fall Line sandhills, or in other areas of the coastal plain near the coast, where this habitat is found particularly along stream borders. These sandhill areas are characterized not only by their coarse white sand with its accompanying extreme aridity, but also by a typical plant association. The distinctive members of this vegetation-habitat complex are the wire grass *Aristida stricta* Michx., the Turkey oak *Quercus laevis* Walt. (= *Q. Catesbaei* Michx.), and the longleaf pine *Pinus australis* Michx. f. (= *P. palustris* Mill.).² Throughout the

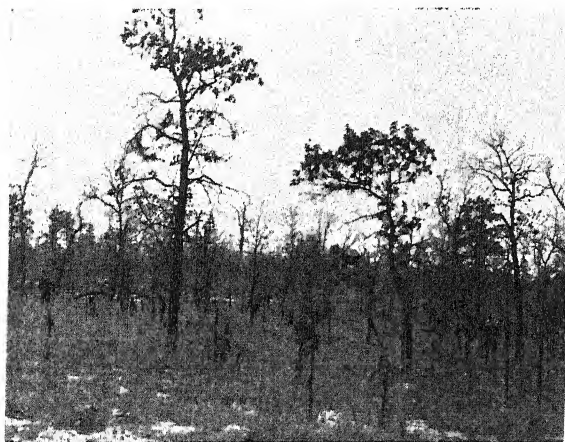


Fig. 4. Habitat of *C. graminea*. View of a "sandhill" area in Bryan County, Georgia, where autotetraploids were collected. For a list of other plants collected at this station see text. Photograph taken by Mr. Ivan Tompkins in February.

southeastern region of the Coastal Plain from North Carolina to Florida the dominant members of this association remain the same, although certain of the other less characteristic species vary from place to place. A good description of this plant community as it occurs in the sandhill region of the southwestern area of the Coastal Plain in North Carolina is given by Wells and Shunk (1931).

To furnish some idea of the plants found associated in the field with *graminea*, table 3 lists some of the species observed in representative areas where collections of this species were made. The first of these areas was visited on June 1, 1940. The locality is one typical of most of the North Carolina "sandhill country" and is located about two miles southeast of Spout Springs. Diploid plants of *graminea* were collected at this spot. The second locality is on

² In all matters of nomenclature Small's Manual (1933) will be followed.

the east bank of the Canoochee River in Bryan County, Georgia, an area typical of the sandhills of the lower terraces of the Coastal Plain nearer the sea. It was visited on September 11, 1939, at which time tetraploid plants of *C. graminea*, which had apparently finished blooming for the year, were collected. A photograph of this area, taken by Mr. Ivan Tompkins on February 25, 1940 (fig. 4), gives a good idea of the physical appearance of the sandhill type of habitat. The third locality is about five miles east of Aiken, South Carolina, on U. S. highway 1 where several tetraploids and one hexaploid plant of *graminea* were collected. This area was visited on June 28, 1940, and again on April 11, 1941. The soil at this spot, though quite sandy, is considerably grayer in color than that in the more typical areas and contains more humus. The vegetation is also somewhat different, being less xeric in character. As will be noted in table 3, oaks other than *Q. laevis* were present. The two other species noted, *Q. Margaretta* and *Q. marilandica*, occur in the xero-mesic phase of the sandhill succession as shown by Wells and Shunk (1931). The collection of tetraploids of *graminea* in this habitat and at other similar stations throughout the range of this form is of considerable interest, since it indicates a greater ecological amplitude of the tetraploids. Even though both forms are characteristically found in the typical sandhill habitat, the diploids are apparently more restricted in their ecological distribution.

One particular advantage in using *C. graminea* in a study such as the present one should be mentioned here, for it is a direct consequence of the ecological peculiarities of the species. Since this plant is a member of a vegetation-habitat complex restricted largely to sterile soils, for the most part unfit for agriculture, the species has been subjected to almost no human interference. This fact permits an interpretation of the past history of the species to be made from present observations with considerable assurance that it has not been unduly complicated by recent artificial environmental changes. In this respect the situation is more satisfactory in *Cuthbertia* than in *Tradescantia*, for the natural habitats of many of the species in the latter genus have been seriously modified by man (Anderson and Hubricht, 1938). Such a condition tends to distort the results obtained from a study of "natural" populations.

GEOGRAPHICAL DISTRIBUTION OF THE CYTOLOGICAL FORMS.—The first collections of *C. graminea* were all tetraploids, but, during the summer of 1939, diploids were discovered by Dr. Carl Swanson and Dr. Thomas Kerr in the sandhill country of the North Carolina Coastal Plain. As more plants were collected and examined cytologically, it began to appear that the diploid and tetraploid forms occupied distinct areas. In order to be certain of this difference, and to delimit, in so far as possible, the respective ranges of the two forms, an extensive collection of living material was made on the South Atlantic Coastal Plain and grown at the Arnold Arboretum.

TABLE 4. Data on collections of *Cuthbertia graminea*. Under location, the distance and direction from the point of reference are usually given; numbers refer to federal (U. S.) and state highways along which many of the collections were made. Abbreviations of collectors' names are explained in the text.

Collection name	No. of plants	Chrom. no.	Locality	Collector
Aberdeen	2	2x	Aberdeen, N. C., 3 mi. S U. S. 15	K., G.
Fort Bragg	7	2x	Fort Bragg, N. C., State 87	K., G.
Harnett	3	2x	Spout Springs, N. C., 2 mi. SE	K., S., G.
Hoke	3	2x (1=3x)	Hoke County Line, N. C., N U. S. 15	K., G.
Marston	1	2x	Marston, N. C., 3 mi. SW U. S. 1	K.
Moore	1	2x	Moore County Line, N. C., S State 211	K., G.
Raeferd	2	2x	Raeferd, N. C., 2 mi. E U. S. 15A	K., G.
Rankin	3	2x	Lillington, N. C., 7 mi. S U. S. 15A	R.
Hoffman	1	2x	Hoffman, N. C., 3 mi. NE U. S. 1	K.
Robeson	2	2x	Rennert, N. C., Pocosin 1 mi. E	K., G.
Sanatorium	2	2x	Sanatorium, N. C.	K., G.
Scotland	1	2x	Laurinburg, N. C., 10 mi. N	K., G.
Pearce	3	4x	Fayetteville, N. C. (near)	R.
Vander	1	4x	Vander, N. C.	K., G.
Cumberland	4	4x	Fayetteville, N. C., 5 mi. E State 24	K., S., G.
Fayetteville	3	4x	Fayetteville, N. C., 5 mi. S	R.
Rex	3	4x	Rex, N. C. (pocosin rim near)	K.
St. Paul's	2	4x	St. Paul's, N. C., 4 mi. E State 20	K.
Page's Lake	3	4x	Bladen County, N. C. (NW corner) State 20	K.
Kenan	8	4x	Kenanville, N. C., 8 mi. S	K., S.
Burgaw	5	4x	NE Cape Fear R. near Burgaw, N. C.	K., S.
White Lake	3	4x	Near White Lake, N. C.	R.
Bladen	1	4x	Black River at Beatty's Bridge, N. C.	R.
Warwich Bay	2	4x	Lumberton, N. C., 10 mi. SE	K.
New Hanover	3	4x	Carolina Beach, N. C., 3 mi. N	H. J. Oosting
Carolina Beach	2	4x	Carolina Beach, N. C., ½ mi. N	K., S.
Brunswick	1	4x	Southport, N. C., 4 mi. W	K., G.
U. S. 1	3	4x	Monetto, S. C., 2 mi. S U. S. 1	G.
Lexington	4	4x	Lexington, S. C., 5 mi. W U. S. 1	G.
Columbia	1	4x	Columbia, S. C., 2 mi. N U. S. 1	G.
Blaney	3	4x	Blaney, S. C., 3 mi. N U. S. 1	G.
Camden	2	4x	Camden, S. C., 4 mi. N U. S. 1	G.
Edmund	1	4x	Edmund, S. C., 3 mi. N State 215	G.
Monetto	3	4x	Monetto, S. C., 3 mi. S U. S. 1	G.
Aiken	9	4x ^a (1=6x)	Aiken, S. C., 5 mi. E U. S. 1	G.
South Fork	2	4x	Aiken, S. C., 8 mi. E State 215	G.
Richmond	6	4x	Augusta, Ga., 10 mi. W	G.
Tarbox	1	4x	Brookgreen, S. C.	F. G. Tarbox, Jr. ^b
Godfrey	1	4x	Georgetown, S. C., 10 mi. N U. S. 17	R. K. Godfrey
Georgetown	3	4x	Georgetown, S. C.	R. K. Godfrey
Sylvania	2	4x	Sylvania, Ga., 8 mi. N State 24	G.
Screvan	7	4x	Newington, Ga., 6 mi. E	E.
Adrian	3	4x	Adrian, Ga., 1 mi. W U. S. 80	G.
Ohoopce	1	4x	Ohoopce R., Emanuel Co., Ga., U. S. 80	G.
Graymont	1	4x	Graymont, Ga., 1 mi. E U. S. 80	G.
Canoochee	4	4x	Canoochee R. near Groveland, Ga.	E., G.
Tifton	4	4x	Irwin County, 9 mi. NE of Tifton, Ga.	E.
Trail Ridge	6	4x ^a	Moniac, Ga., 12 mi. S	E.
Tisonia	1	4x	Tisonia, Fla., 1 mi. N U. S. 17	G.
Marietta	3	4x	Marietta, Fla., 2 mi. W U. S. 90	G.
Clay	2	4x	Maxville, Fla., 5 mi. S State 13	G.
Firetower	2	4x	Highland, Fla., 3 mi. S State 13	G.
Boswick	2	4x	Boswick, Fla., 1 mi. N	G.
Alachua	1	4x	Gainesville, Fla., 5 mi. E State 14	G.
Gainesville	5	4x	Gainesville, Fla.	W. A. Murrill
Putman	2	4x	Hawthorn, Fla., 7 mi. E State 14	G.
Interlachen	2	4x	Interlachen, Fla., 1 mi. W State 14	G.
Palatka	1	4x	Palatka, Fla., 4 mi. W State 14	G.
Lake	1	6x	Lake County, Fla.	W. A. Murrill

^a 1=4x+1. ^b Data from Anderson and Sax (1936).

The data on these collections are given in table 4. A name has been assigned to each collection. The number of plants in the collection is then given, followed by the chromosome number, the approximate geographical location, and the collector's name. In some cases chromosome counts have been obtained from preserved material also. In all but two instances, however, these preserved collections were made along with living plants. The collections are arranged with the localities of the diploids first, alphabetically, followed by the localities of the tetraploids in geographical order, going from north to south. To simplify the table, abbreviations for certain collectors' names have been used. These are as follows: Dr. Thomas Kerr=K.; Dr. Carl Swanson=S.; Mr. Don Eyles=E.; Mr. H. A. Rankin=R.; the writer=G. To all those who have collected specimens, the writer is greatly indebted, and especially to the four mentioned above who have expended considerable time and effort in so doing. As yet, no search has been made for stations of *graminea* in the Piedmont province adjacent to the Fall Line, but if the plants do occur in this province they must be relatively rare, as indicated by the citations of specimens by Anderson and Woodson (1935).

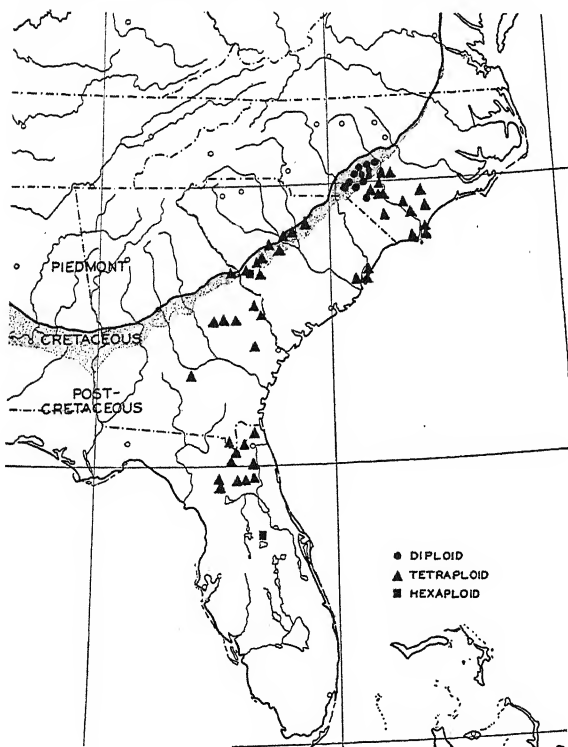


Fig. 5. The distribution of the three cytological forms of *C. graminea* on the South Atlantic Coastal Plain. The heavy black line indicates the position of the Fall Line, the inner limit of the Coastal Plain sediments. The stippled area indicates the extent of surficial deposits of Cretaceous age; the unstippled area east and south of the Fall Line represents surficial deposits of post Cretaceous, primarily Pleistocene, age. Based on Goode Base Map No. 209E. By permission of the University of Chicago Press.

The localities where the collections were made have been indicated on the accompanying map (fig. 5). It is immediately clear that the diploids are restricted to a relatively small area in North Carolina, the tetraploids occupy a much more extensive area throughout the South Atlantic Coastal Plain, and the hexaploids have been found at only two widely separated stations. It also appears that the diploids and tetraploids do not occur in the same area. With a single exception (plants collected near Rennett in Robeson County, North Carolina) all the diploids are confined to the typical sandhills along the western edge of the Coastal Plain in North Carolina. In no case have diploids and tetraploids been found growing together in the field. A single triploid plant was obtained at the Hoke County Line station in North Carolina. It is worthy of note that this locality is apparently just on the boundary between the diploid and tetraploid areas. The hexaploid from the Aiken (South Carolina) station, however, was found growing in the middle of a large tetraploid population.

DISCUSSION.—It is clear from the preceding evidence that the advent of autopolyploidy has profoundly modified the composition of the natural population known taxonomically as *Cuthbertia graminea*. The autotetraploids are found to differ from the ancestral diploids in a number of important respects. Morphologically they exhibit gigantism, and there are also preliminary data indicating that they have a greater variability. Physiologically they appear to be more vigorous. Cytologically they are more variable, as shown by the presence of extra-chromosomal types. Unpublished studies also indicate a greater frequency of structural changes in the chromosomes of the tetraploids. Factors such as the above are presumably the basis for the distributional differences characterizing the two forms. From an ecological point of view, there is evidence suggesting that the tetraploids can occupy more diverse habitats than the diploids. Further, there is the striking difference in geographical distribution.

From the cytological and distributional data which are now available, together with a knowledge of the geology of the region under discussion, it is possible to obtain a fairly clear picture of the past history of the *graminea* complex and to make at least a preliminary evaluation of the rôle of autopolyploidy in its development.

Briefly, the geological history of this area is as follows: The entire South Atlantic Coastal Plain is underlain by sedimentary deposits ranging in age from Upper Cretaceous to Recent. The older deposits are those immediately adjacent to the Fall Line, which marks the limit of the crystalline Piedmont province. Deposits successively younger in age occupy the region between the Fall Line and the coast, a very considerable proportion of these being terraces of Pleistocene origin. On the map (fig. 5) the extent of the surficial deposits which are considered to have originated primarily from the weathering of the Upper Cretaceous sediments is indicated.

The data on which these limits are based have been obtained from Stephenson (1912), Cooke (1936), and Fenneman (1938). The North Carolina sandhills, which were formerly included in the Lafayette formation (Stephenson, 1912), are now considered to be composed of the weathered residual products mainly of the Tuscaloosa formation (Upper Cretaceous) and subordinately of the Paleocene (Cooke, 1936; Stephenson, 1940). This region adjacent to the Fall Line has probably not been submerged by the sea since early Tertiary at the latest.

There is a notable correlation between the geological evidence and the geographical facts. The diploids occupy an area geologically older than that in which most of the tetraploids occur. The obvious interpretation is that the North Carolina sandhills represent entirely or in part the original area occupied by the ancestral diploids and their descendants, and that the derived autotetraploids, being more vigorous and aggressive, have been almost entirely responsible for the colonization of lands as they became available following the retreat of the sea within relatively recent times.

Another question of considerable interest concerns the possibility that the more vigorous tetraploids have been able to replace the original diploids over some or a considerable portion of their range. Under such circumstances the present diploid area would represent only a certain fraction of that formerly occupied by diploids. It is not possible at present to answer this question definitely, but certain preliminary evidence indicates that such a replacement may have occurred. The first collections of *C. graminea* were tetraploids from the Pleistocene terraces of North Carolina and Georgia. When the diploid area was discovered in North Carolina, it was assumed that diploids would also be found in the Fall Line sandhills of South Carolina and Georgia. However, fairly extensive recent collections from Augusta to Columbia and Camden have revealed only tetraploids in this area. As yet no specimens have been secured between Camden, South Carolina, and the North Carolina sandhills, so that it is not yet possible to define the southern limit of the diploid area. On the basis of the present evidence, however, it seems possible that the tetraploids may have been able to supplant the diploids in a part of their original range along the Fall Line. On the other hand there may be certain geological or ecological differences between these two adjacent sandhill regions which account for the limited range of the diploids, but as yet no such differences have been detected. More field study and the collections are necessary before this point can be determined. Such evidence will be of further value, since it may make possible an estimate of the time of origin, geologically, of the tetraploids.

It is not yet possible to draw definite conclusions as to the evolutionary significance of the autohexaploids, since only two of these have been discovered so far. The fact that they have proved so rare, and that the two individuals were collected at wholly

unrelated localities (Aiken, South Carolina, and central Florida) indicates that they are probably sporadic forms which may occur with a low frequency in any tetraploid population. This low population frequency itself suggests that their increased chromosome number has somehow resulted in decreased selective advantages, at least in competition with their tetraploid ancestors. However, it is not impossible that the hexaploids are of very recent origin and may be in the process of becoming established as an element in the natural population at the present time. As yet this possibility cannot be eliminated and a decision must await further evidence.

A difference in range between diploid and autotetraploid intraspecific races has been noted previously in several plant species. An example which is similar in several respects to the one being discussed here is that reported by Manton (1934, 1937) for *Biscutella laevigata* L. In this species the diploid forms ($2x=18$) are restricted to the unglaciated river valleys of Central Europe, whereas the tetraploids ($4x=36$) occur in nearly the whole area occupied by the Alpine Ice sheet during the Glacial Period. The evidence indicates that the diploids are waning inter-glacial or pre-glacial relics, and are not expanding their territory, whereas the tetraploids are vigorous post-glacial immigrants to many of their present districts and may still be spreading. An autohexaploid form ($6x=54$) also occurs in northern Spain and appears to be separated geographically from the rest of the complex.

The tetraploids of *B. laevigata* also differ from the diploids in being cytologically unstable, almost 20 per cent having somatic chromosome numbers other than the expected thirty-six. Further, they have the capacity to propagate themselves vegetatively as is often the case in forms possessing unbalanced chromosome complements. By way of contrast *C. graminea* has been found to be relatively stable cytologically, since, as indicated in table 2, less than 3 per cent of the plants from a sample of similar size to that taken in *Biscutella* were aneuploid types. A difference in the methods of collecting the data in these two cases should be pointed out, however. The chromosome numbers found in *graminea* were all obtained from mature plants collected in the field. These plants had consequently been subjected as seedlings to natural selection. For *Biscutella* most of the counts were obtained from seedlings grown in the greenhouse from seeds collected in nature. The difference in the cytological stability is very probably a real one, however, and might be expected, since the degree of unbalance in a $4x+1$ aneuploid having a base number of nine would presumably be less than in one with a base number of six. It is also worthy of note as a correlation with the lower frequency of aneuploids that propagation in *C. graminea* occurs almost exclusively by seed, and not by vegetative means.

Intraspecific autopolyploidy has been demonstrated in several species of the *virginiana* complex of the closely related genus *Tradescantia* (Ander-

son and Sax, 1936; Anderson and Woodson, 1935; Anderson, 1937). In this case the area of the combined ranges of the tetraploids is considerably greater than that of the diploids, and the diploid forms occupy a region which is geologically old and has been continuously open for plant habitation. It is significant that even though considerable genetic divergence separates the two genera *Cuthbertia* and *Tradescantia* the intrageneric evolutionary patterns of the two seem to be basically similar.

In *Tradescantia* it is apparent that the spread of the more successful tetraploids has been primarily to the northeast and northwest from a center of distribution near central Texas. Conversely, in *Cuthbertia graminea* this spread has been largely southward. These observations are of some interest in connection with studies (cf. Tischler, 1935; Flovik, 1940) showing that the percentage of species with high chromosome numbers is greater in the flora of Northern than of Southern Europe. Many of these species are undoubtedly allopolyploids, but it also seems true that in cases of intraspecific autopolyploidy the tetraploids have as a rule a more arctic or alpine distribution than the diploids (Müntzing, 1936). These observations have been interpreted as indicating a relation between climate and distribution of the tetraploids, the explanation being that these presumably more vigorous types could occupy areas in which the diploids were not able to survive. However, it seems clear from the two cases cited that the direction of spread is probably not conditioned as much by the presence of a more rigorous climate as by the availability of new territory for plant occupancy. It is apparently of no great importance whether territory becomes available for plant occupancy as a result of the retreat of glaciers to the north or of seas to the south; in any case the tetraploids, if more vigorous, will do the colonizing. The restriction in northward spread of the tetraploid *C. graminea* has apparently been due in large part to the lack of habitats adapted to the relatively strict ecological requirements of the species.

CONCLUSIONS.—Autopolyploidy has been a factor of major importance in the evolution of the *Cuthbertia graminea* complex. The profound physiological consequences of polyploidy have enabled the tetraploids to effect a great expansion of the range of the species ecologically, and especially geographically, by colonizing new territory, most of which has become available for plant occupation in relatively recent (Pleistocene) times. The ancestral diploids have been left behind as a small relic population in an area geologically old (Cretaceous). Further, there is some evidence suggesting that the tetraploids may have displaced the diploids from a part of the latter's original range. If such a process of replacement has actually occurred and continues, the result should be the complete elimination of the diploids, and eventually the species would be represented in nature only by the derived polyploids.

It is not yet possible to evaluate fully the significance of the rare and sporadic occurrence in natural

populations of individuals showing still higher degrees of autopolyploidy (hexaploids). The fact that the hexaploids have not become a distinct and numerically significant element in the population may indicate that the advantages resulting from an increase in chromosome number are diminished or lost in this species complex above the tetraploid level. The two hexaploids so far discovered would then presumably represent fortuitous cases of autopolyploidy which might be expected to occur occasionally in almost any plant population. However, another interpretation of the hexaploids seems possible. It may be that their high degree of polyploidy is of very recent origin, and that this cytological form is even now in the process of becoming established as a new and important element in the population.

SUMMARY

Intraspecific natural autopolyploidy is shown to occur in *Cuthbertia graminea*. Wild populations of this taxonomic species include diploids, autotetraploids, and autohexaploids. The base number of the species is $x=6$; four of the chromosomes of the haploid set have subterminal, and two median centromeres.

The genus *Cuthbertia* is rather closely related to the *virginiana* species complex of *Tradescantia*. However, the striking differences in chromosome morphology of the two groups imply a considerable genetic divergence, and this is supported by purely genetic evidence, since numerous attempts to make hybrids between species in the two groups have failed.

A comparative study of the diploids and autotetraploids of *C. graminea* has revealed that the two types differ strikingly in several respects. These differences are as follows:

(a) Morphologically the tetraploids exhibit considerable gigantism; physiologically they are characterized by greater vigor, both in the field and in the greenhouse.

(b) Cytologically the tetraploids are more variable. Two plants out of seventy-five examined (2.7 per cent) were aneuploids ($4x+1$), whereas no aneuploids have been found among the diploids.

(c) Although both forms are largely restricted to xeric sandhill habitats of the Coastal Plain, there is evidence that the tetraploids possess greater ecological amplitude.

(d) Perhaps the outstanding difference is one of geographical distribution. Extensive collections and examinations of living plants have shown that the diploids are restricted to a relatively small area in the Fall Line Sandhills of southern North Carolina, whereas the tetraploids occupy an extensive area throughout the remainder of the Coastal Plain.

The ecological restriction of *C. graminea* to xeric sandhill habitats makes this species particularly suitable for an analysis of historical changes in natural populations, since there has been little modification of these economically almost worthless areas as a result of human interference.

A correlation of evidence from cytology, geographical distribution, and geology, makes it possible partially to reconstruct the past history of the species. The area occupied by the diploids is geologically old (Cretaceous and Paleocene), and these plants, which on cytological evidence are the original form, are to be considered a relic population. The much larger area occupied by the tetraploids, however, is for the most part geologically young (Pleistocene), and it seems clear that the derived and more vigorous tetraploids have effected a great expansion of the range of the species by colonizing new territory as it became available for plant occupancy. The

advent of autotetraploidy must be considered an event of major importance in the evolution of the *Cuthbertia graminea* complex.

Autohexaploids ($6x=36$) appear to be of rare and sporadic occurrence in this species as only two individuals have been discovered so far. The evidence is as yet insufficient to justify a full evaluation of their significance. Two possible interpretations of the hexaploids are presented, however, and discussed briefly.

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NOTES ON SOME BROWN ALGAE FROM THE MONTEREY PENINSULA, CALIFORNIA ¹

Gilbert M. Smith

FOR THE past five years the writer has been engaged in the preparation of a monograph on the marine algae of the Monterey Peninsula. This peninsula lies at the southern end of Monterey Bay, California, and includes that portion of the shore line between Monterey and the Carmel River. The present paper deals with certain taxonomic problems that have arisen in connection with the brown algae of this area. Most of these problems arise from the fact that phycologists working on marine algae of the Pacific Coast of North America have based their conclusions chiefly on herbarium specimens. Study of living plants, especially as they are found growing *in situ*, has revealed additional information contributing to an interpretation of certain of these controversial species.

In studying the marine algae of the Monterey Peninsula I have received assistance from various

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quarters. The Department of Botany at the University of California has offered every facility for studying their extensive collections of marine algae from the Pacific Coast of North America. Miss Rosalie Weikert has supplied information concerning Californian algae of the Anderson collection in the Phycological Herbarium of the New York Botanical Garden, and Dr. David H. Linder has answered numerous inquiries concerning material in the Farlow Herbarium. Professor G. J. Hollenberg has kindly prepared the Latin diagnosis for *Dictyonopsis*. My thanks are also due to the Research Committee of Stanford University for a grant which made possible the preparation of figures 5-13 by Mrs. Carl F. Janish.

ECTOCARPUS VARIABILIS (SAUNDERS) COMB. NOV.—In 1893 Rosenvinge found certain specimens of *Ectocarpus confervoides* with peculiar uniseriate reproductive organs which he interpreted as a row

of unilocular sporangia. Kuckuck (1894, p. 234), the next phycologist to mention these structures, interpreted them as abortive plurilocular sporangia. Sauvageau (1896) came to the same conclusion and

assumed that the abnormality was due to some unknown parasite. Saunders (1898, p. 155; pl. 23), apparently unaware of previously published observations, called them a uniseriate row of unilocular sporangia and held that their production in a row occurred with sufficient regularity to make it one of the characters in describing his *E. confervoides* forma *variabilis*. He states that they are "mostly on the same filament with the plurilocular sporangia, but much less common" whereas in his figures each of the filaments bears only one kind of reproductive organ. Setchell and Gardner (1925, p. 414), although recognizing Saunders' forma *variabilis* as distinct, are strongly inclined towards Sauvageau's interpretation of the uniseriate organs as abortive plurilocular organs.

E. confervoides forma *variabilis* Saunders is not uncommon on the Monterey Peninsula. It is epiphytic on several kelps growing between the 0.0 and -1.5 foot tide levels, but is usually found either on *Laminaria Andersonii* Farlow or on *Lessoniopsis littoralis* (Farlow and Setchell) Reinke. In *E. confervoides* f. *variabilis*, as found on the Monterey Peninsula, there are many erect branched filaments growing from a prostrate filamentous base. Some of the erect filaments bear uniseriate organs only, a considerably larger number bear only pluriseriate organs. Less than half a dozen filaments have been found with both uni- and pluriseriate organs.

Study of living uniseriate organs this past summer shows that the cells have protoplasts containing a few discoid chromatophores much lighter in color than those in vegetative cells (fig. 3-4). The lighter color of the chromatophores is thought to be a normal rather than a pathological condition. Many of the uniseriate organs were found to have discharged their contents through a single pore in the lateral wall (fig. 3). Here there was a complete discharge of the cell contents instead of a retention of some of the cytoplasm as described by Sauvageau. Unfortunately, actual discharge of the contents was not observed. The failure to observe the discharge cannot be ascribed to immaturity of material or to unfavorable conditions in the laboratory because pluriseriate organs in the same collections liberated swimmers in abundance.

There is both negative and positive evidence for thinking that the uniseriate organs are plurilocular in nature rather than a row of unilocular sporangia. On the negative side is the fact that examination of hundreds of living uniseriate organs failed to reveal a single instance where there was a cleavage of the protoplast. If the uniseriate fertile cells are unilocular sporangia, the failure to find cleavage stages is difficult to explain because, as is well known, cleavage stages are of frequent occurrence when unilocular sporangia are present in abundance. On the positive side the following reasons indicate that the fertile row is a plurilocular organ. (1) The number of enlarged fertile cells in a row is increased by transverse division just as in early development of a plurilocular sporangium. (2) A new fertile row may

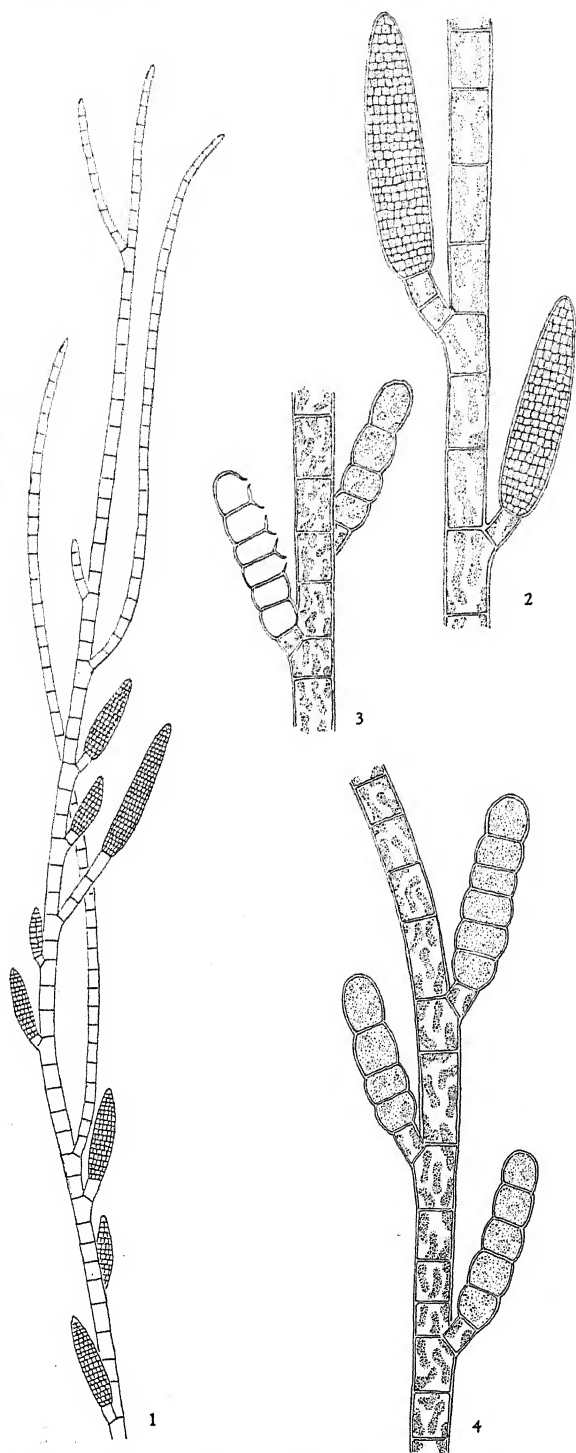


Fig. 1-4. *Ectocarpus variabilis*.—Fig. 1. Branch with pluriseriate organs. $\times 100$.—Fig. 2. Pluriseriate organs. $\times 325$.—Fig. 3-4. Uniseriate organs. $\times 325$.

develop within an old empty one in just the same manner as a new plurilocular sporangium develops from the stalk cell of an old empty one. (3) There may be a development of aberrant reproductive organs which are partly uniseriate and partly pluriseriate. Sauvageau (1896, p. 143, fig. 4) interprets such structures as abnormal plurilocular organs. Saunders (1898, pl. 23, fig. 5) gives a figure of one of them but makes no comment concerning its nature. Several of these abnormal organs have been found on Monterey Peninsula material, and all of them were on filaments bearing uniseriate organs. This indicates that the pluriseriate portion is reversionary and that the uniseriate row is plurilocular in nature.

Although it seems quite clear that the uniseriate organs are plurilocular in nature, there is no evidence indicating whether they are gametangia or sporangia. The gametangial or sporangial nature of the pluriseriate organs is also uncertain. In the case of the latter organs, repeated attempts were made this past summer to obtain gametic union by mixing swimmers from different thalli but with negative results. However, even this is inconclusive because the alga may be heterogamous and the pluriseriate organs the male gametangia.

The regular production of two kinds of plurilocular organs by *E. confervoides* forma *variabilis* is so distinctive a character that it warrants elevation of the form to the rank of a species.

Ectocarpus variabilis (Saunders) comb. nov. (figs. 1-4)

E. confervoides forma *variabilis* Saunders 1898, p. 155; pl. 23. Setchell and Gardner 1925, p. 414; pl. 46, fig. 7. Saunders in Phyc. Bor.-Amer. No. 526.

E. confervoides Rosenvinge [not of (Roth) Le Jolis]. Rosenvinge 1893, p. 188; fig. 21. Kuckuck 1894, p. 234. Sauvageau 1896, p. 140; fig. 1-4.

Thalli in gregarious tufts 0.5-2.0 cm. tall; epiphytic and attached by prostrate rhizoidal branches which do not penetrate the host; erect branches gradually attenuated, sparingly branched and with the branching predominately alternate; the cells up to 30 μ broad, with a length 1-3 times the breadth, and containing several short band-shaped chromatophores; plurilocular sporangia (?) of two sorts and usually borne on separate erect filaments; one type of plurilocular sporangium multiseriate, fusiform to narrowly ovoid, 10-15 cells broad in surface view, and with a pedicel of one to several cells; the other type uniseriate, with 2-15 barrel-shaped cells, and with a 1- to 3-celled pedicel; multiseriate sporangia 15-35 μ broad, 75-180 μ long, and with the cells about 2 μ broad; uniseriate sporangia 15-35 μ broad, 70-200 μ long, and with the cells 15-35 μ broad.

Type from Point Pinos, Pacific Grove, California. On the Pacific Coast of North America ranging from Puget Sound to central California (Carmel Bay). Also known from Greenland, Helgoland, and France.

THE CENTRAL CALIFORNIA SPECIES OF MACROCYSTIS.—Along the coast of central California, and

especially on the Monterey Peninsula, there are extensive beds of *Macrocystis* both in the intertidal zone and where the water is twenty to sixty feet deep. One or two other deep-water kelps along the coast, as *Nereocystis Luetkeana* (Mert.) Post. and Rupr., may have occasional stray individuals growing in the intertidal zone, but in the case of *Macrocystis* the intertidal individuals are so numerous and grow in such extensive stands that they cannot be considered stragglers from deeper water. At localities where deep-water beds of *Macrocystis* grow directly off shore from intertidal beds the interval between the two is barren of *Macrocystis* or contains only a few stray individuals. Examples of this on the Monterey Peninsula are to be found at Mussel Point and at Pebble Beach. Here the beds of intertidal plants end rather abruptly at the -0.5 foot tide level and the depth of water on the shore side of deep-water beds is approximately twenty feet.

In their treatise on the brown algae of the Pacific Coast of North America, Setchell and Gardner (1925) consider the intertidal and deep-water plants distinct species primarily distinguishable from each other by structure of the holdfast. *M. integrifolia* Bory, the shore species, is described as having a prostrate, dichotomously branched, flattened, rhizome-like holdfast with haptera arising along the lateral margins. *M. pyrifera* (L.) C. A. Agardh, the deep-water species, is described as having a large massive holdfast with a large number of dichotomously branched haptera. Later, as a result of intensive study of holdfasts, Setchell (1932) comes to the conclusion that there are no fundamental differences between deep-water and intertidal specimens as found between Sitka (Alaska) and Point Conception (central California). He holds that *M. integrifolia* is the only species along this long stretch of coast line. He also thinks that all *Macrocystis* from Point Conception southward to where it disappears from the North American algal flora near Magdala Bay in Baja California (Mexico) is *M. pyrifera*.

Since the Monterey Peninsula lies within the range where Setchell thinks that all individuals should be referred to *M. integrifolia*, special attention has been given to the question of differences between deep-water and intertidal specimens. More than twenty separate beds of intertidal plants have been studied on the Monterey Peninsula, and in every one of them the plants were found to have prostrate rhizomatous holdfasts; never the massive conical holdfast so frequently found on specimens of *Macrocystis* taken from deep water. The holdfasts of local intertidal plants are similar to those figured by Setchell and Gardner (1925, pl. 62) rather than to those figured by Howe (1914, pl. 19).

Intertidal plants usually grow where they are continually swept by the surf, whereas deep-water plants grow in relatively quiet water. From this it might be argued that the characteristic differences between holdfasts of the two are due to environmental factors. To test this possibility intertidal plants were collected at Pebble Beach and at Cypress



Fig. 5-8, $\times \frac{1}{2}$.—Fig. 5-6. Branch tip and holdfast of *Macrocystis integrifolia*.—Fig. 7-8. Terminal blade and holdfast of *Macrocystis pyrifera*.

Point. Pebble Beach borders a small land-locked cove where the water is always quiet; Cypress Point is a rocky headland against which the surf is continually pounding. Holdfasts of plants from these two markedly different habitats are essentially the same and show that the distinctive shape of the holdfast of an intertidal plant is not modified by agitation of the water.

Setchell's (1932) conclusion that there are no fundamental differences between holdfasts of intertidal and deep-water specimens of *Macrocystis* as found north of Point Conception was based in large part upon a comparison of adult plants. He was unable to make comparative studies of relatively young plants because he did not have specimens from deep water to compare with juvenile plants he had collected from the intertidal zone. During the past summer juvenile plants from the intertidal area have been compared with juvenile plants from deep water. The intertidal plants were collected at Mussel Point. The deep-water plants were dredged from a depth of 25 to 30 feet at a submerged reef about a quarter-mile northeast of the municipal pier at Monterey. Some of the intact deep-water plants were attached to pieces of rock broken from the reef by the dredge; others were torn from the reef by the dredge.

Little need be said concerning holdfasts of juvenile intertidal plants, since these have been fully described and adequately illustrated by Setchell (1932). As he has shown, the basal portion of the stipe becomes decumbent, distinctly flattened, and has development of haptera restricted to the two lateral margins of the flattened rhizome-like portion (fig. 6). Deep-water plants at a corresponding stage of development have an entirely different holdfast. Here the basal portion of the stipe is erect (never decumbent), and cylindrical or very slightly compressed. The haptera develop on all sides of the base of a stipe (fig. 8), instead of distichously as in intertidal plants. Holdfasts of juvenile deep-water plants are quite similar to those figured by Skottsberg (1907, fig. 98, 105-109) for juvenile plants of *M. pyrifera* as found in the Antarctic. When Monterey Peninsula deep-water plants become older there is a development of additional, radially disposed, haptera above those already formed, and production of additional haptera continues at successively higher levels until they are formed above the first forking of a stipe. Holdfasts of local deep-water plants in which haptera are being produced above the first dichotomy of a stipe bearing a strong resemblance to Setchell's (1932, pls. 40-42) illustrations of holdfasts of plants dredged at a depth of 16 to 20 fathoms near San Diego in southern California.

The blades borne at regular intervals along the stipe vary so greatly in length, breadth, dentation, bullation of the lamina, and in shape of the basal pneumatocyst, that phycologists have not utilized blade characters in distinguishing species. However, there are constant differences between terminal blades of intertidal and deep-water Monterey Peninsula plants. As is well known, the terminal blade of

Macrocystis produces new blades by splitting asymmetrically in the basal meristematic region where it joins the stipe. In the case of an intertidal plant the blade terminating a well-developed stipe is narrowly falcate, has a length five to fifteen times the breadth, and rarely has more than six young blades in progressive stages of development (fig. 5). On the other hand, terminal blades of the off-shore plant are broadly falcate, rarely have a length more than five times the breadth, and usually have ten to twenty young blades in successive stages of development (fig. 7). A similar difference is to be found in published figures of terminal blades of *Macrocystis*. Illustrations of intertidal plants from Chile (Howe, 1914, pls. 22-23) and from twenty miles south of San Francisco, California, (Setchell, 1932, pl. 34-35) show the narrow falcate terminal blades characteristic of intertidal plants on the Monterey Peninsula. The terminal blades of *M. pyrifera* as figured by Hooker (1847, pl. 171, fig. A), by Harvey (1862, pl. 202), and by Skottsberg (1907, fig. 110, 112) are of the broadly falcate type and with a considerable number of young blades incompletely cut away from the terminal blade.

The value of these terminal blade characters as a means of distinguishing between littoral and sublittoral plants was tested by requesting a colleague to make two random collections of terminal blades; one from the intertidal bed at Mussel Point, the other from a deep water bed a short distance out from Mussel Point. He was asked to put an identification tag on each blade and then to mix the two collections. The writer then segregated the mixed collection into two groups, one assumed to have been collected in the intertidal region, the other from deep water. When the results were verified by checking the numbered identification tags it was found that the source of every one of the fifty or more blades had been determined correctly. There is one point that should be noted in connection with this striking demonstration of differences between terminal blades of plants from the two habitats. In the case of the deep-water plant my colleague who collected the material worked from a rowboat and gathered terminal blades floating horizontally at or just beneath the water's surface. Thus the only terminal blades gathered by the collector were those on well-developed stipes with a length of 5 m. or more. The results might not have been so conclusive if juvenile deep-water plants had been available, since their terminal blades are somewhat narrower than those of mature plants.

There is also a constant difference between the length of local intertidal and deep-water plants. The intertidal plant is generally but 3-5 m. long, the deep-water plant frequently has a length of 15-20 m. The greatest length thus far found for Monterey Peninsula specimens of the deep-water plant is 26 m., a length considerably below the maximum of 47.5 m. recorded by Frye, Rigg and Crandall (1915) for plants found elsewhere along the coast of California. The question as to whether these constant

differences in size are due to specific differences or to environmental factors cannot be answered with certainty. In several other marine algae of the local

flora, specimens collected from the sublittoral region are regularly larger than those from the intertidal region. *Nereocystis Luetkeana* is a striking ex-

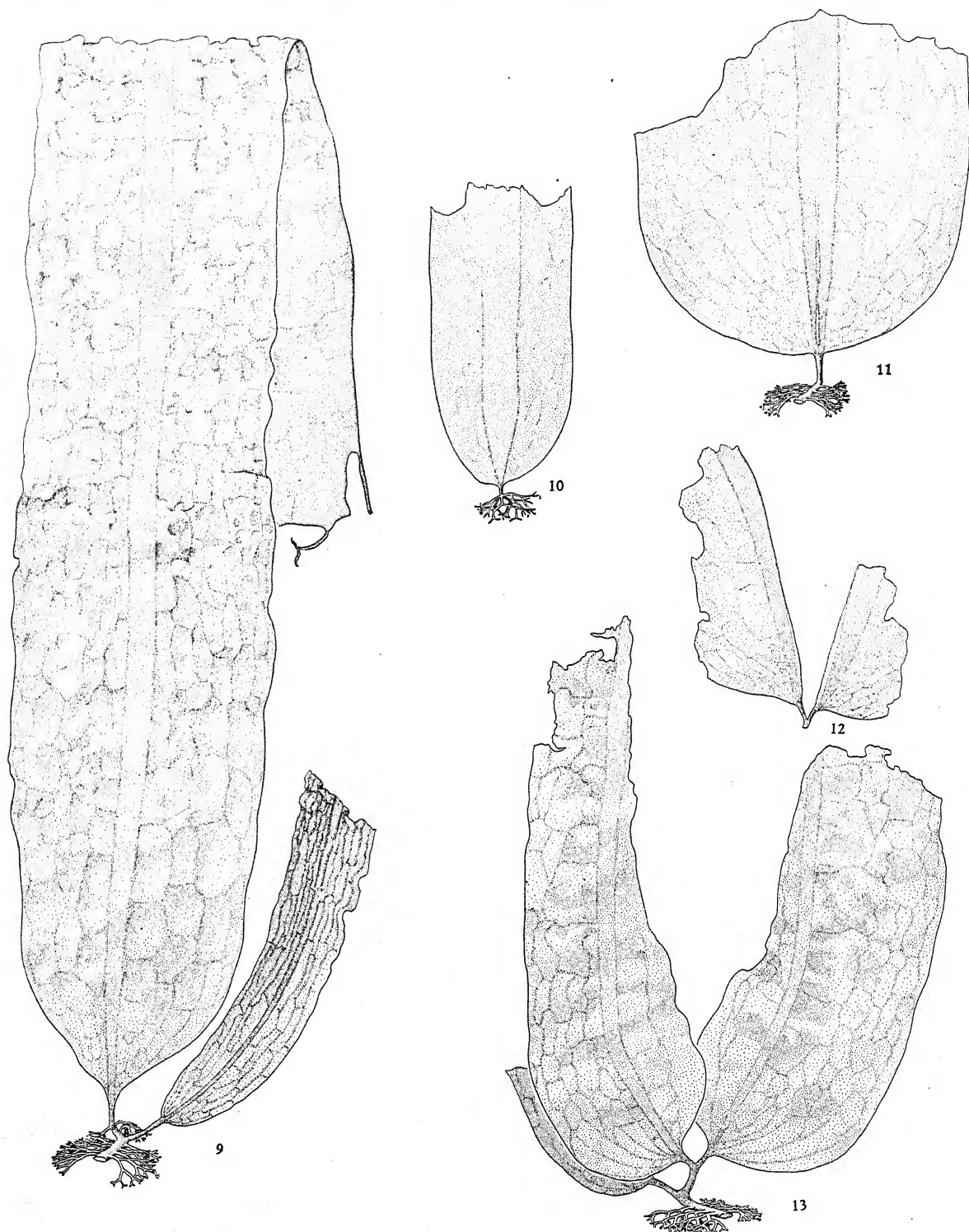


Fig. 9-13. *Dictyoneuropsis reticulata*.—Fig. 9, 11-13, $\times \frac{1}{4}$.—Fig. 10, $\times \frac{1}{2}$.—Fig. 9. Mature plant.—Fig. 10. Young plant.—Fig. 11. Beginning of longitudinal splitting of midrib.—Fig. 12-13. Stages in formation of new half-blades.

ample of this. Here the stipe is 1–2 m. long if the alga is growing in the intertidal zone, but it is up to 35 m. long if the alga is growing in deep water. The experimental demonstration (Hurd, 1916) that depth of submergence is the primary factor affecting length of stipe in *Nereocystis* indicates that differences in length between intertidal and deep-water plants in *Macrocystis* may be due to environmental factors.

Study of *Macrocystis* as found on the Monterey Peninsula shows that there are constant differences between holdfasts and terminal blades of intertidal and deep-water plants, and that these differences are not due to environmental factors. All of the evidence is in favor of the 1925 opinion of Setchell and Gardner where the intertidal and deep-water plants are considered distinct species.

DICTYONEUROPSIS GEN. NOV.—In 1895 Saunders described *Costaria reticulata*, from material collected near Pacific Grove, California. He first found fragments of blades cast ashore on the beach; later he dredged additional fragments from a depth of fifty feet and complete plants from a depth of eight to fifteen feet. Saunders separated *C. reticulata* from other species of *Costaria* because of the single longitudinal rib. The year following Setchell (1896, pp. 46–48), after studying an authentic specimen sent him by Saunders and specimens furnished by C. L. Anderson, came to the conclusion that the alga is a *Dictyoneurum* and merely a form of *D. californicum* Ruprecht, the single species of that genus. Setchell emphasized the fact that certain of Anderson's specimens had a dichotomously branched stipe with each dichotomy terminating in a single blade. From this he assumed that blades of *C. reticulata* split longitudinally in the same manner as those of *D. californicum*. He also laid great stress on the fact that juvenile blades of *D. californicum* have a differentiated axial strip resembling the midrib of *C. reticulata*. Setchell and Gardner (1925, p. 622), after studying the above-mentioned specimens and additional ones from Mrs. J. M. Weeks of Pacific Grove, unreservedly stated that Saunders had described juvenile blades of *D. californicum*.

This past summer *C. reticulata* was rediscovered growing at a depth of twenty to thirty feet near Point Aulon, Pacific Grove; and at a depth of twenty-five to thirty feet on a submerged reef about a quarter-mile northeast of the municipal pier at Monterey. The material dredged at these two localities consisted of a hundred or more fragmentary blades and thirteen intact plants with holdfasts. The latter ranged from very young plants 1.5 cm. tall to adult plants 95 cm. in height. Blades on certain of the intact plants were in various stages of splitting from base to apex and they confirm the correctness of Setchell's inference that the alga multiplies vegetatively in the same manner as *Dictyoneurum*. These plants show that splitting begins in the basal portion of the midrib of a blade (fig. 11), and then extends vertically upward to the blade apex and vertically downward through the erect portion of the

stipe. The two half-blades, each with a half of the original midrib at one side, become widely divergent soon after splitting is completed. Divergence of the two halves of a blade is accompanied by a regeneration of a new half-blade along the recently divided midrib. Regeneration of a new half-blade begins at the base of a midrib (fig. 12) and gradually extends upward (fig. 13). The fact that more than half of the hundred or more fragmentary blades brought up by the dredge had an asymmetrical midrib similar to that figured by Saunders (1895, pl. 7, fig. 1) indicates that complete regeneration of a new half-blade takes a long time.

Setchell's conclusion that Saunders' alga is more closely related to *Dictyoneurum* than to *Costaria* is undebatable. On the other hand, his conclusion that it is identical with *Dictyoneurum californicum* seems unjustified. One of his major arguments for considering the two algae identical is the fact that juvenile blades of *D. californicum* frequently have an axial longitudinal strip closely resembling the midrib of *C. reticulata*. But, as Setchell has already noted (1896, p. 47), there is always more or less reticulation of the pseudo-midrib of *D. californicum*. On the other hand, Saunders states that there are never reticulations on the midrib of *C. reticulata* and the blades collected this past summer confirm his statement. This is clearly evident on living blades. It is not so evident on blades of herbarium specimens because drying of bullations of a midrib make it seem to be reticulate. Setchell and Gardner (1925, p. 622) hold that Saunders found a midrib because he collected primary blades. The fact that every one of the hundred or more blades collected this past summer had a midrib indicates very clearly that all blades of *C. reticulata* have a midrib. Setchell was unable to corroborate Saunders' statement that the midrib portion of *C. reticulata* is about double the thickness of other parts of a blade. Measurements of a few blades dredged from the vicinity of Point Aulon showed the midrib to be 250–350 μ in thickness as against a thickness of 150–190 μ in portions lateral to the midrib.

In addition to the midrib there are several other characters distinguishing *C. reticulata* from *D. californicum*. The interspaces between reticulations in mature blades of the former are 8–30 mm. wide; those of the latter are 5–15 mm. The arrangement of reticulations is also different in the two. In *C. reticulata* the reticulations lie in an irregularly distributed pattern; in *D. californicum* the more or less rectangular reticulations so coincide with one another that there are longitudinal ridges throughout the length of a blade.

Mature blades of the two are similar to the extent that they are approximately the same breadth throughout, but the two differ markedly in shape of the basal portion. The base of a blade of *C. reticulata* is broadly rounded or even cordate; that of *D. californicum* (cf. Setchell and Gardner, 1925, pl. 70) is more or less cuneate. Differences between blade margins of the two are not constant, but blades of

C. reticulata never have the small spine-like projections which are so frequently present in *D. californicum*. There are also differences in size. Mature blades of *C. reticulata* are up to 95 cm. long, with a maximum breadth of 15–25 cm. Those of *D. californicum*, as found on the Monterey Peninsula, are up to 65 cm. long and 4–8 cm. broad.

Holdfasts of the two algae also differ in several respects. The rhizome portion of the stipe of *C. reticulata* is 4–6 mm. broad and 1 mm. in thickness; that of *D. californicum* is 6–15 mm. broad and frequently 2–3 mm. in thickness. Both algae have the haptera restricted to lateral margins of the dichotomously branched rhizomes, but the haptera themselves are different. In *C. reticulata* the haptera are three to five times dichotomous and 25–30 mm. long; on the other alga they are usually but once or twice dichotomous and 8–12 mm. long. *D. californicum* frequently has 50 to 100 blades as a result of eight to ten successive dichotomous splittings at the base of a thallus. Sufficient data are not at hand to state unreservedly that *C. reticulata* forms a smaller number of blades, but in the few intact plants collected last summer there were never more than three successive dichotomous splittings and a resultant formation of eight blades.

The foregoing discussion has shown that Saunders' alga is not a *Costaria*, and that it is a species distinct from *Dictyoneurum californicum*. If the only differences between Saunders' alga and *D. californicum* were in size, shape, and reticulation of blades, and in form and dimensions of rhizome and haptera, one would undoubtedly place *C. reticulata* as a second species of *Dictyoneurum*. The stumbling block is the lack of a midrib in blades of *Dictyoneurum*. In the case of certain other Laminariales found along the Pacific coast of North America, the presence of a differentiated axial strip through the blade has been deemed sufficient justification for recognition of new genera. One example of this is seen in Setchell's (1901, p. 123) differentiation of *Pleuraphycus* from *Laminaria*; another example is Reinke's (1903, p. 25) segregation of *Lessoniopsis* from *Lessonia*. Since "*Costaria reticulata*" seems to bear a similar relationship to *Dictyoneurum* the best solution of the problem seems to be to make it the type of a new genus. An even more important argument for considering *C. reticulata* generically distinct from *Dictyoneurum* is the clean-cut manner in which a new half-blade is regenerated along the exposed margin of a recently divided blade. There is a possibility that increase in breadth of a recently divided blade of *D. californicum* is also unilateral, but there are no coordinating points on the blade to prove that this is the case.

Dictyoneuropsis gen. nov.—Thalli perennial, at first with an unbranched prostrate stipe whose distal end bends upward and terminates in a single sublinear costate blade; decumbent portion of stipe flattened and with haptera along the lateral margins, the blade and erect portion of stipe later splitting longitudinally to form a forked stipe with each

arm terminating in a blade; blades dividing longitudinally through the midrib and regenerating new half-blades from the midrib; surfaces of blades with a coarse reticulum of narrow ridges except along the midrib; unilocular sporangia in irregularly shaped sori on both flattened surfaces of the blade, the sori at times covering the midrib. Gametophytes unknown.

Thalli perennes, primum cum stipo prostrato sine ramis, cuius terminus distalis sursum curvat et in lamina singula et sublineari et costata terminat; parte prostrata stiporum complanata et cum hapteris in marginibus lateralibus; lamina et parte erecta stipi recentius longitudinaliter diffidente et stipum furcatum faciente, quaque furca in lamina terminante; laminis per costam longitudinaliter transdividentibus, semilamina nova secundum costam regenerata; superficiebus laminarum reticulum crassum. Fastigiorum angustiorum praeter secundum costam habentibus; sporangium unilocularibus soris irregulares in utroque superficie laminarum formantibus; soris interdum costam vestientibus; plantis sexualibus ignotis.

Dictyoneuropsis reticulata (Saunders) comb. nov. (fig. 9–13).

Costaria reticulata Saunders 1895, p. 58, pl. 7.

Thalli up to 95 cm. tall; prostrate portion of stipe up to three times dichotomous; the dichotomies 4–6 mm. broad, 1 mm. in thickness; with haptera along the lateral margins, the haptera three to five times dichotomous and 25–30 mm. long; mature blades linear, slightly narrowed at upper end, broadly rounded at base; reticulation of blade restricted to portions lateral to midrib, but entire blade at times bullate; blades up to 90 cm. long, with a maximum breadth of blade 15–25 cm., midrib 1.5–3.5 cm. broad.

Growing in the sublittoral zone and at a depth of eight to fifty feet.

Type locality "Monterey Bay near Pacific Grove, Cal." Type specimen in Farlow Herbarium, Harvard University.

Also known from Point Aulon, Pacific Grove; and from one-fourth mile northeast of the municipal pier, Monterey, California.

SUMMARY

In *Ectocarpus variabilis* (Saunders) comb. nov. the uniseriate row of large fertile cells is to be interpreted as a plurilocular organ rather than as a row of unilocular sporangia.

Deep-water and intertidal plants of *Macrocytis* as found northward from Point Conception, California, have been variously considered two distinct species (*M. pyrifera* and *M. integrifolia*) and a single species (*M. integrifolia*). Comparison of holdfasts and of terminal blades of the two shows that they should be considered two species.

Costaria reticulata Saunders is made the type of a new genus, *Dictyoneuropsis*. This genus multiplies vegetatively in the same manner as *Dictyoneurum* but differs in the manner of development subsequent

to longitudinal splitting of a blade. The presence of a midrib is another reason for considering it distinct from *Dictyonereum*.

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STUDIES IN THE GENUS SCIRPUS L. V. NOTES ON THE SECTION ACTAEOGETON REICH.¹

Alan A. Beetle

THE SECTION Actaeogeton Reich. of the genus *Scirpus* (Cyperaceae) falls into the subgenus *Aphylloides* Beetle (1940), being related to the sections *Schoenoplectus* Palla (*S. americanus* and its allies) and *Lacustres* Clarke (*S. lacustris* and its allies). It is easily distinguishable from both of these by the possession of black or gray-brown achenes which are marked with horizontal ridges. Only two species and two varieties out of the fifteen species and three varieties comprising the section Actaeogeton occur in North America, all the others being confined to the Old World.

In the preparation of this paper the section has been studied as a whole. The key includes all species that the material available seemed to justify. Notes on species that are not North American are included only where a nomenclatural change was necessary for the proper annotation of herbarium specimens.

In the preparation of this paper material in the herbaria of the following institutions has been consulted: Gray Herbarium (G), Harvard University; University of California, Berkeley (UC); New York Botanical Garden; University of West Virginia; California Academy of Sciences; Dudley Herbarium, Stanford University; Jessup Herbarium, Dartmouth College; Missouri Botanical Garden (MO), St. Louis; Rocky Mountain Herbarium, University of Wyoming; Field Museum of Natural History, Chicago; and the author's personal collection. In the interest of brevity the source of the specimens except in the case of types, will not be further specified.

¹ Received for publication May 19, 1942.

TAXONOMIC TREATMENT.—Section Actaeogeton Reichenbach, *Icones Florae Germanicae et Helveticae* 40. 1846. Annual or rarely perennial herbs; culms triangular or often very obscurely angled, green, smooth; sheaths usually bladeless or nearly so; outer involucre bract appearing as a continuation of the culm, green, smooth, glabrous; bristles present or absent; stamens 3; style 2- or 3-fid; achene marked by horizontal ridges, dark-gray brown or black, trigonous or lenticular, apiculate. (Type species: *Scirpus mucronatus* L.)

KEY TO THE SPECIES

Plants perennial

At least some spikelets rayed

Involucre bract short and inconspicuous, 1-3 cm. long

1. *S. brachyceras*

Involucre bract long, appearing as a continuation of the culm, 7-9 cm. long.....2. *S. inclinatus*

All spikelets sessile

Culms often 5 mm. thick at base

Involucre bract short, rarely up to 7 cm. long; bristles present.....3. *S. mucronatus*

Involucre bract long, never less than 15 cm. in length; bristles absent.....4. *S. articulatus*

Culms slender, seldom over 2 mm. thick at base

6. *S. erectus*

Plants annual

Involucre bract solitary

Achene lenticular or slightly plano-convex

Scales appressed at maturity

Spikelets 1-5; achenes olive brown

Spikelets paired.....7. *S. erectus* var. *wallichii*

Spikelets not consistently paired.....6. *S. erectus*

Spikelets many; achenes black.....9. *S. bucharicus*

Scales spreading at maturity.....13. *S. vohemarensis*

- Achene sharply trigonous
 Achene obscurely roughened
 Involucral bract strictly erect, $\frac{1}{2}$ to $\frac{2}{3}$ length of plant.....5. *S. fistulosus*
 Involucral bract often divergent or incurved, not more than $\frac{1}{4}$ length of plant
 Culms rigid, yellow-green, spikelets 1-many; upper sheaths bladeless
 11. *S. smithii* var. *williamsii*
 Culms flaccid, dull green; spikelets 1-3; upper sheaths blade-bearing.....10. *S. smithii*
 Achene prominently horizontally ridged
 Scales of spikelets spreading at maturity
 12. *S. roylei* ✓
 Scales of spikelets appressed at maturity
 Involucral bract 1-3 dm. long.....8. *S. schoofii*
 Involucral bract up to 1 dm. long
 14. *S. supinus* ✓
 Secondary involucral bract present
 Sheaths blade-bearing
 Some spikelets rayed.....15. *S. saximontanus*
 All spikelets sessile.....14. *S. supinus*
 Sheaths bladeless
 Achene dull gray-brown.....16. *S. reductus*
 Achene black
 Achene obscurely ridged, mostly not apiculate
 17. *S. uninodis* ✓
 Achene prominently ridged, apiculate
 18. *S. uninodis* var. *hallii*

1. *SCIRPUS BRACHY CERAS* Hochst. ex A. Rich. Tent. Fl. Abyss. 2: 496. 1847-51. Africa.
2. *SCIRPUS INCLINATUS* Aschers. & Schweinf. ex Boiss. Fl. Orient. 5: 381. 1884. Africa.

This species has usually been referred to as *Scirpus corymbosus* Roth, Nov. Pl. Sp. 28. 1821 which is based on *Isolepis corymbosa* Roth, ex R. & S. Veg. Syst. 2: 110. 1817. There are, however, two earlier, validly published homonyms, not only that of Linnaeus (1756, Cent. Pl. 2: 7) which refers to a species of *Rhynchospora* but also that of Forskal (1775, Fl. Aegypt. Arab. 14) which is a synonym of *Scirpus maritimus* L. *Scirpus inclinatus* is here considered the first available name.

3. *SCIRPUS MUCRONATUS* L. Sp. Pl. 1: 50. 1753. Of wide distribution in the Old World.

Scirpus mucronatus is absent from the Americas. The report of it by Britton (1888) was based on the erroneous identification of exceptionally large specimens of *S. smithii* var. *williamsii*. This same conclusion was reached by Long (1918), and agreed to by Britton, as indicated by correspondence in the Herbarium of the New York Botanical Garden.

4. *SCIRPUS ARTICULATUS* L. Sp. Pl. 1: 47. 1753. Southern Asia and Australia.
5. *SCIRPUS FISTULOSUS* Forsk. Fl. Aegypt. Arab. 14. 1775. Africa.
6. *SCIRPUS ERECTUS* Poir. in Lam. Encyc. Meth. Bot. 6: 761. 1804. Asia and the Hawaiian Islands.
7. *SCIRPUS ERECTUS* var. *wallichii* (Wight) Beetle comb. nov. China and India. *Scirpus wallichii* Nees ex Wight, Contr. Bot. Ind. 112. 1834.

This entity was well characterized by Wight whose description emphasized its important characteristics: "involucro erecto monophyllo longo tetra-

gonis spiculis subgeminis squamis ovatis acutis margine albo-membranaceis" Here, as in *S. erectus*, the style has been observed to be either 2- or 3-fid in the same spikelet. Although the variety is readily separable from *S. erectus*, its achenes are identical. Since speciation has been indicated by distinct achenes in all other cases in this section the varietal status is employed for this entity.

8. *SCIRPUS schoofii* Beetle sp. nov.

Herba caespitosa, radicibus fibrosis; culmus 3-7 dm. altus, erectus, viridis, compresso-angulatus vel teres, basi vaginatus, vaginis superioribus laminis mucronatis 1-2 cm. longis praeditis; involucrum recte apicale 1-3 dm. longum; spicae sessiles, 8-20, 7-10 mm. longae, 3 mm. latae, rubiginosae, squamae fertiles 3 mm. longae, 1.5 mm. latae, mucronatae; setae aut 6 aut saepe nullae; stylus trifidus; achenium 2 mm. longum, 1 mm. latum, triquetrum, mucronulatum, nigrum, transverse rugosulum. *Scirpo erecto* valde affinis.

Fibrous rooted annual; culms 3-7 dm. high, erect, green, slender, very obscurely angled or terete; sheathed at the base, the upper sheaths bearing rudimentary blades 1-2 cm. long; involucral bract single, strictly erect, 1-3 dm. long, slightly dilated at the base; spikelets sessile, 8-20, ovate-acute, 7-10 mm. long, 3 mm. broad, reddish-brown; sterile outer scale present; fertile scales 3 mm. long, 1.5 mm. broad, mucronate-tipped, bristles present or absent; stigma 3-fid; achene 2 mm. long, 1 mm. broad, trigonous, strongly marked with horizontal ridges, mucronate, black.

Type from China, Yunnan, *E. E. Maire* 6706 (UC).

Named for Howard William Schoof.

9. *SCIRPUS BUCHARICUS* Roshev. ex Komarov. Fl. U. R. S. S. 3: 55 (570). 1935. Asia.
10. *SCIRPUS SMITHII* Gray, Man. Bot. N. U. S. ed. 5. 563. 1867.

Scirpus smithii var. *levisetus* Fassett, Rhodora 23: 42. 1921.

Cespitose annual; culms 1-2 dm. high, erect or sometimes prostrate spreading, dull green, slender, terete or angled, sheathed at the base, upper sheaths bearing blades up to 1 dm. long; involucral bract single, up to 1 dm. long, erect or usually curved over the spikelets; spikelets usually 1-3, ovate-acute, sessile, 4-8 mm. long, 3 mm. broad; scales 3 mm. long, smooth, green, slightly mucronate-tipped; bristles absent or 2-6, equaling or exceeding the achene; style 2-fid; achene 2 mm. long, 1.5 mm. broad, shiny black, obscurely horizontally ridged, plano-convex or obscurely trigonous, apiculate.

Type from New Jersey, "shore of the Delaware below Redbank, opposite mouth of the Schuylkill, July, 1865," *C. E. Smith* (G).

QUEBEC: *Fernald* 2514. MAINE: *Fassett* 26 (type of *Scirpus smithii* var. *levisetus*). NEW HAMPSHIRE: *Pease* 19253. VERMONT: *E. & C. E. Faxon* in 1881. MASSACHUSETTS: *Weatherby* in 1911. RHODE ISLAND: *E. & C. E. Faxon* in 1880. CONNECTICUT: *Bissell* in 1910.

NEW YORK: *Haberer 1181*. PENNSYLVANIA: *Fogg 5328*. VIRGINIA: *Shull 214*.

The specimens here treated as *Scirpus smithii* form a kind of trap very frequently met in the Cyperaceae, one that catches a too literal taxonomist. Such closely allied species as *S. articulatus* and *S. erectus* may be distinguished from the closely related *S. supinus* with its sharply trigonous achene. Such characters as these suddenly become jumbled with great inconsistency in specimens of *S. smithii*, where either trigonous or lenticular achenes may be found either with or without bristles. Even the style may be inconsistently 2- or 3-fid within the same spikelet. Hence, the above characters can only serve to separate isolated specimens within this group. The more consistent characters of the obscurely roughened seed coat, growth habit, and vegetative scale and sheath shape must be relied on to define specific limits. Here again judgment must be exercised for a more unstable condition could hardly be singled out than the triangular as opposed to terete culms emphasized in Gray's Manual, ed. 7.

No entities more difficult of separation have been encountered than in the *S. debilis* Pursh-*S. smithii* complex. In the study of this group, after adopting as usual the view that a species is guilty until proven innocent, it was found that the proposed subdivisions of *S. smithii* failed to warrant independent taxonomic status. Apparently *S. smithii* is no more than an aquatic extreme of *S. debilis*. Very frequently in Cyperaceae the juvenile stages exhibit features which become lost in fully mature plants. The first sheaths formed, for example, may have long linear blades whereas the later sheaths are bladeless. From observations of sedges growing under various environmental conditions, it is suggested that plants growing in excess of water will longer retain juvenile features, e.g., blade-bearing sheaths. Typical *S. smithii* never has more than three spikelets and always has an erect but slightly incurved involucre bract. Furthermore, there is a correlation between the two characters. All specimens of *S. debilis* with three or fewer spikelets have an erect involucre bract, but those with four or more spikelets have a reflexed bract, which appears to be merely a mechanical forcing back due to the position of the fourth spikelet. *Scirpus smithii* has a more terete culm, a duller green cast, a slightly longer involucre bract, somewhat more flaccid culms, a more compressed achene that matures slightly earlier, scales more readily deciduous, and smaller spikelets than *S. debilis*. Of these characters no one seems constant, and many intermediate conditions exist. The characters are, however, clustered together in a definite pattern which allows the marking off of one side of the variation pattern as *S. debilis*, the other as *S. smithii*, and leaves the middle a no-man's land. This would seem to be an excellent series for the study of species origin. *S. smithii* suggests an incipient species, a slowly specializing complex diverging from typical *S. debilis*.

Inasmuch as *S. smithii* and *S. debilis* intergrade morphologically it is possible to draw the line between them at more than one point. A somewhat narrower interpretation of *S. smithii* than has generally been applied has been adopted here because it gives that species a more readily characterized distribution. This change of interpretation includes a change in relation for *S. smithii* var. *setosus*, it now falling in the synonymy of the *debilis* complex. *S. smithii* var. *levisetus* Fassett, characterized as having "bristles 2-6, barbless or rarely subscabrous," differs in no important respect from the type of *S. smithii*.

11. *SCIRPUS smithii* var. *williamsii* (Fernald)
Beetle comb. nov.

Scirpus debilis Pursh, Fl. Am. Sept. 1: 55. 1814, not Lam., Tabl. Encyc. Meth. 1: 141. 1791.

Scirpus debilis var. *williamsii* Fern. Rhodora 3: 252. 1901.

Scirpus smithii var. *setosus* Fern. op. cit. 1901.

Like *Scirpus smithii* but usually more robust; culms up to 6 dm. tall, bright green; the involucre bract often strongly reflexed; spikelets 1-12, bristles absent, or as many as six, occasionally strongly barbed; achene somewhat more strongly trigonous.

Type from Massachusetts, sandy shore of Massapoag Lake, Sharon, September 7, 1901, E. F. Williams and M. L. Fernald (G).

MAINE: *Fernald* in 1894. NEW HAMPSHIRE: *Wheeler* in 1904. VERMONT: *Eggleston & Jones 1726*. MASSACHUSETTS: *Fernald 16372*. RHODE ISLAND: *Reynolds 621*. CONNECTICUT: *Woodward* in 1910. NEW YORK: *Svenson 4695*. NEW JERSEY: *Woolman 128*. PENNSYLVANIA: *Bartram 1133*. MARYLAND: *Blake 8900*. DISTRICT OF COLUMBIA: *Bebb 10*. VIRGINIA: *Fernald & Long 10970*. WEST VIRGINIA: *Greenman 69*. NORTH CAROLINA: *Wiegand & Manning 634*. SOUTH CAROLINA: *Clausen & Trapido 3669*. GEORGIA: *Small* in 1893. OHIO: *Moseley* in 1896. KENTUCKY: *Svenson 4419b*. TENNESSEE: *Svenson 6867*. ALABAMA: *Vasey* in 1878. MICHIGAN: *Hanes 3695*. INDIANA: *Deam 53494*. ILLINOIS: *Mead* in 1845 (type of *Scirpus smithii* var. *setosus*). WISCONSIN: *Fassett & McLaughlin 12196*. MINNESOTA: *Grant 3294*.

If *Scirpus smithii* is considered to be specifically distinct from *S. debilis* of Pursh the latter will have to receive a new name because it has an earlier homonym.

12. *SCIRPUS roylei* (Nees in Wight) Beetle comb. nov. India.

Isolepis roylei Nees in Wight, Contr. Bot. Ind. 107. 1834.

Scirpus roylei is commonly referred to as *Scirpus quinquefarius* (Buch.-Ham. ex Wallich Cat. 121. 1828). The latter name was originally published as a *nomen nudum*, however, and seems not to have been validated before 1869-70 (Boeckler in Linnaea 36: 701), and almost certainly not before Wight validly published for Nees both *Isolepis*

roylei and *I. lupulinus* in 1834. *Isolepis roylei* is first in order. A new combination of the second name (*Scirpus lupulinus*, Roshev. ex Komarov, Fl. U. R. S. S. 3: 53. 1935) was recently made but it is a later homonym of *Scirpus lupulinus* Spreng. (Fl. Hal., Mant. 30. 1811). It, therefore, appears necessary to make a combination on *Isolepis roylei* and to treat this species as *Scirpus roylei* (Nees in Wight) Beetle.

13. *SCIRPUS VOHEMARENSIS* Cherm. Bull. Soc. Bot. France 68: 423. 1921. Madagascar.

14. *SCIRPUS SUPINUS* L. Sp. Pl. 1: 49. 1753. Europe.

15. *SCIRPUS SAXIMONTANUS* Fern. Rhodora 3: 251. 1901.

Fibrous rooted annual; culms 1–3.5 dm. high, erect, green, terete or obscurely angled, sheathed at the base, the upper sheaths often bearing blades 3–12 cm. long; outer involucre bract 5–10 cm. long, erect or incurved; second involucre bract below the spikelets, to 3 cm. long; spikelets 2 to many, both sessile and rayed; scales with hyaline margins, fimbriate, 3.5 mm. long, 3 mm. broad, the green midrib excurrent; bristles absent; stigma 3-fid; achene trigonous, dark gray-brown, prominently horizontally ridged, apiculate, 2 mm. long, 1 mm. broad.

Type from Colorado, Weld County, Greeley, E. L. Greene, September 20, 1872 (G).

OHIO: Pickaway Co., Bartley & Pontius in 1936.

SOUTH DAKOTA: Brown Co., Thorner in 1892; Bangor, Thorner in 1892, Griffiths in 1895. NEBRASKA: Kennedy, Bates in 1892; Holt Co., Tolstead 41432; Perkins Co., Tolstead 41426; Fillmore Co., Tolstead 41425; Franklin Co., Tolstead 41424, 41428, 41429; Kearney Co., Tolstead 41430. KANSAS: Rooks Co., Bartholomew in 1889. TEXAS: C. Wright; Cibola, Lindheimer; Cameron Co., Runyon 2660; Hildalgo Co., Clover 867 & 897; Bexar Co., Neally 90, Metz in 1931. COLORADO: Weld Co., bank of La Poudre, Greene in 1872. MEXICO: Lower California, Comodo Viejo, Brandegee in 1888; San Luis Potosi, Schaffner in 1877.

16. *SCIRPUS REDUCTUS* Cherm. Bull. Soc. Bot. Fr. 68: 423. 1921. Africa.

17. *SCIRPUS UNINODIS* (Delile) Boiss. Fl. Orient. 5: 380. 1884. Eurasia.

18. *SCIRPUS uninodis* var. *hallii* (Gray) Beetle comb. nov.

Scirpus hallii Gray, Addenda, Man. ed. 2. 1863.

Scirpus supinus var. *hallii* Gray, Man. Bot.

N. U. S. ed. 5, 563. 1867.

Fibrous rooted annual; culms tufted, strictly erect, up to 4 dm. high, 1–1.5 mm. thick, very obscurely triangular or terete, green, flexuous, soft,

sheathed at the base, the sheaths green, prominently mucronate tipped; reduced spikelets consisting of a single flower enclosed in a sheath often found at base of culms; outer involucre bract 3–12 cm. long, strictly erect, smooth, a second 0.5–1 cm. long, often present below the spikelets; spikelets 1-many, sessile or short-rayed, 3–15 mm. long, ovate-acute, green drying to a pale straw brown, scales 3 mm. long, keeled, mucronate tipped, appressed at maturity; bristles present or absent; style 2-fid; achene 1.5 mm. long, 1 mm. broad, plano-convex, black, apiculate, strongly marked with horizontal ridges.

Type from Missouri, St. Louis, September, 1845. G. Engelmann (G).

MASSACHUSETTS: W. Boott in 1876; Pl. Exsicc. Gray. 143. ILLINOIS: Menard Co., Athens, E. Hall in 1861. FLORIDA: Curtis 3118, Harper 38. GEORGIA: Harper 1203. TEXAS: F. Lindheimer in 1847–48.

The North American material differs from the Eurasian only in having slightly longer spikelets, and more strongly ridged and more prominently beaked achenes. That Gray recognized the close similarity of *Scirpus uninodis* and *S. uninodis* var. *hallii* is indicated by the following note in his handwriting on a sheet of the former in the Gray Herbarium: "has solitary flower in axil!—my *S. supinus hallii*—having a basal flower!"

SUMMARY

For the section Actaeogeton of *Scirpus* (Cyperaceae) fifteen species and three varieties are recognized. Distributions, descriptions, and complete synonymy are given for North American entities only. *Scirpus schoofi* from China is described as new.

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DEVELOPMENT OF THE GAMETOPHYTES AND FERTILIZATION IN CAMASSIA¹

Frank H. Smith

THERE HAVE been two brief descriptions of the development of the megagametophyte of *Camassia*. Leffingwell (1930) reported what is now known as the *Adoxa*-type of megagametophyte development for *C. quamash* (Pursh) Greene. Fagerlind (1941) corrected this interpretation in a short paper describing the normal-type of development for this and two unidentified species of *Camassia*. In neither case was there any investigation of the microgametophyte or fertilization. *Camassia* provides unusually favorable material for the study of both male and female gametophytes and fertilization and has been so used in this laboratory for some time. This paper was prepared, then, with two purposes in mind: first, to give more extensive descriptions of the development of the gametophytes, especially the later development of the megagametophyte, and of the fertilization processes than are now available; and second, to recommend the use of *Camassia* in the classroom to demonstrate the development of the Angiosperm megagametophyte. In connection with the latter aim, an attempt is made to indicate the approximate rate of development of the flowers in relation to that of the gametophytes, and the time at which material should be collected for given stages.

The lily has been used almost universally in general botany laboratories to demonstrate megagametophyte development, probably because of the ease of obtaining and manipulating the material. It was known from the first that *Lilium* did not show the normal-type of development, but the original interpretation could be given to beginning students without too much difficulty or confusion. Following the appearance of the paper by Cooper (1935), however, it was recognized that the difficulties associated with the use of *Lilium* in the general laboratory were greater than had been supposed. Some attempts have been made to replace lily with some species showing the normal-type of development, such as certain of the composites, but these have not been favorably received. If a species is to be suitable for mass production of slides to show development of the megagametophyte, numerous flowers in various stages should be readily available; a fairly large number of ovules, properly oriented, should be present in each ovary; and no great difficulties should appear in fixing and staining the material. *Camassia* meets all these requirements. Bulbs may be obtained from almost any nursery; the flowers are borne in a raceme, the number varying up to thirty or more; and cross sections of the ovary give median sections of most of the ovules.

MATERIAL AND METHODS.—*Camassia Leichtlinii* (Baker) Wats. is widely distributed in the lowlands throughout the Pacific Coast states. It differs from

C. quamash in that it is usually larger, has regular flowers and perianth segments which twist about the ovary as they wither, shortly after fertilization occurs. Both species are rather widely cultivated. The two species are very similar with regard to the development of the gametophytes and fertilization. For this investigation only material of *C. Leichtlinii* is considered. This was collected, usually at weekly intervals, from plants growing in the field. Of the various fixing reagents used, Karpechenko's modification of Navashin's solution gave by far the best results for all stages. Small ovaries were fixed intact but the larger ones had their tips removed to expose the ovules. A low vacuum was used at times, but the only advantage seemed to be that it aided in infiltration with paraffin by removing most of the air from the locules. Night temperatures were fairly low during the period in which the material was collected. When ovaries were fixed in the early afternoon, there were comparatively frequent division figures in all stages. After the material was immersed overnight in the fixing solution, it was washed in running water for two hours. Dehydration and infiltration were accomplished by using a much shortened tertiary butyl alcohol schedule. After washing, the material was placed in 15 per cent ethyl alcohol for fifteen minutes, 30 per cent alcohol for thirty minutes, 50 per cent alcohol for one hour, one-half absolute alcohol and one-half Shell tertiary butyl alcohol for one hour, Shell tertiary butyl alcohol for two hours, and in three-fourths Shell tertiary butyl alcohol and one-fourth xylol for two hours. Paraffin chips were added to this mixture, and the unstoppered vials were placed in the paraffin oven for four hours. The material was then passed through two changes of a mixture of one-half Tissuemat and one-half 53° paraffin at five- to ten-hour intervals. This same mixture of Tissuemat and paraffin was also used as the embedding medium. Longer periods may be used as a matter of convenience.

Sections to show the reduction divisions and development of the megagametophyte as far as the two-nucleate stage were cut at 10 microns. Sections to show the stages from the four-nucleate condition to the early seven-celled gametophyte were cut at 12–14 microns, and the mature megagametophyte was cut at 16–18 microns. To follow the development of the male gametes in the pollen tube during their passage down the style, longitudinal sections were cut at 8 microns.

Of the staining procedures tried, the most desirable from the standpoint of results and ease of manipulation was safranin and aniline blue. The slides were stained in 1 per cent aqueous safranin for about twelve hours, rinsed in water, then in 95 per cent alcohol containing 0.5 gram of picric acid per 100 cc. for five to ten seconds. Each slide was then flooded twice, quickly, with 95 per cent alcohol containing three to four drops of ammonia per 100

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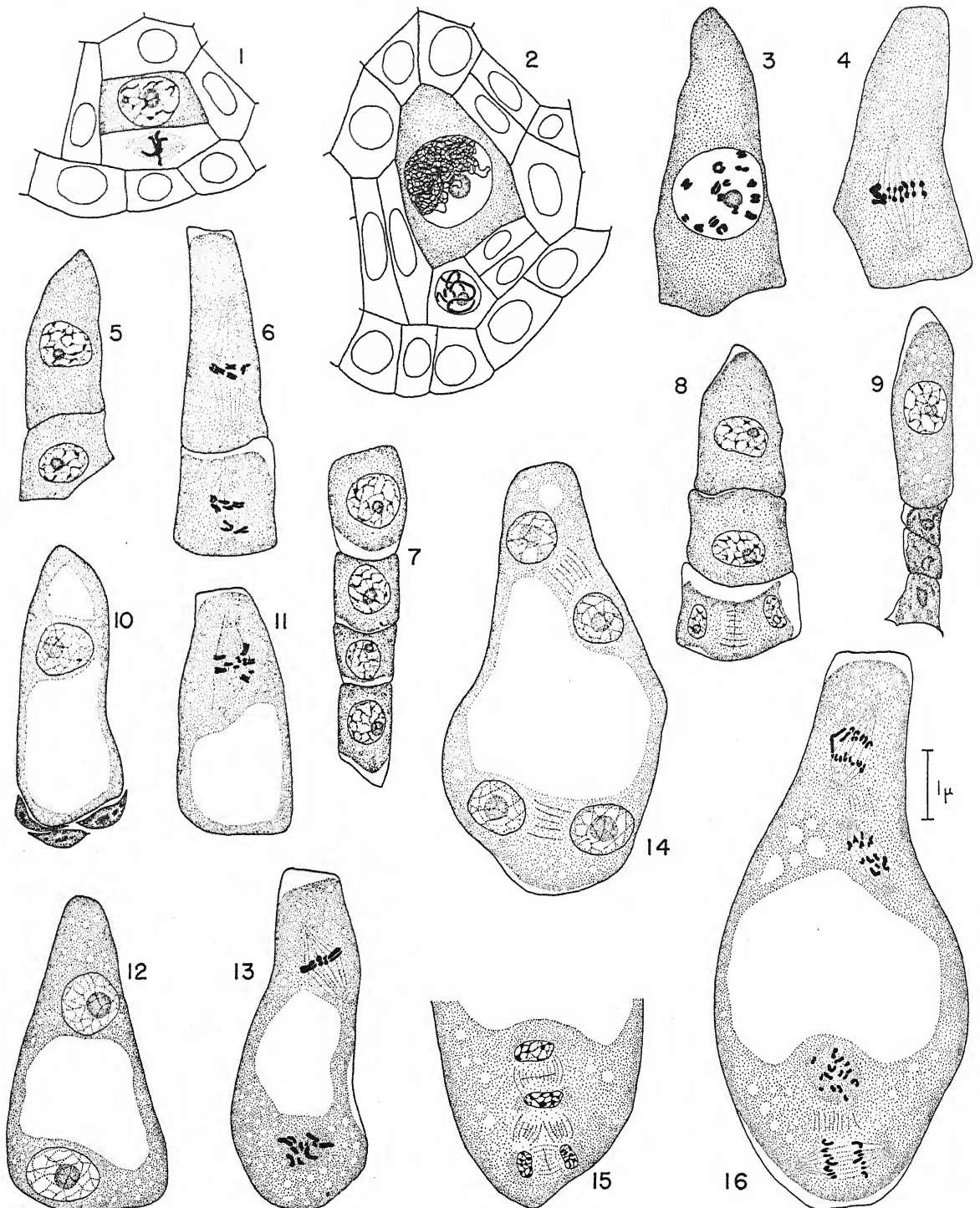


Fig. 1-16. Megasporogenesis and young megagametophyte. The micropylar end is directed downward in all figures and all are shown at the same magnification.—Fig. 1. Young megaspore mother cell and first division of the parietal cell.—Fig. 2. Megaspore mother cell at leptotene, with surrounding cells.—Fig. 3. Diakinesis.—Fig. 4. Metaphase of first reduction division.—Fig. 5. Dyad.—Fig. 6. Second reduction divisions, metaphase in the chalazal cell and pre-metaphase in the micropylar cell.—Fig. 7. Linear tetrad of megaspores.—Fig. 8. T-tetrad of megaspores.—Fig. 9. Functional and disintegrating megaspores.—Fig. 10. Megaspore after formation of vacuoles, and disintegrating megaspores.—Fig. 11. Pre-metaphase of first nuclear division.—Fig. 12. Two-nucleate stage.—Fig. 13. Metaphase of second nuclear divisions.—Fig. 14. Four-nucleate stage showing persistent fibers of second divisions.—Fig. 15. Cell plate formation between micropylar nuclei after third divisions.—Fig. 16. Anaphases of the third divisions.

cc., rinsed in fresh 95 per cent alcohol and then flooded with 1 per cent aniline blue in 95 per cent alcohol. The length of time in aniline blue is not critical. Thirty seconds to one minute was the time generally used. The stain was then rinsed off quickly with absolute alcohol and the aniline blue differentiated with clove oil. With a little practice, undesirable slides may be eliminated at this point but this must be done rather quickly, before too much blue is removed by the clove oil. The slides were then passed through three changes of xylol over a period of one hour and mounted in gum dammar. Material showing fertilization stages must be passed more slowly through the 95 per cent alcohols to remove more of the safranin than is desirable for other stages.

DEVELOPMENT OF THE MEGAGAMETOPHYTE.—A single archesporial cell is evident in the young ovule before the integuments have started to form. It may be distinguished from the surrounding cells by its larger nucleus and densely granular cytoplasm. In later stages it becomes somewhat larger than the surrounding cells of the nucellus. A single parietal cell is soon cut off, which divides, usually antichinally, very soon after it is formed (fig. 1). The inner cell enlarges rapidly, increasing mostly in length, and functions as the megaspore mother cell. Soon the nucleus enters the prophase of the first reduction division. By the time the nucleus of the megaspore mother cell is in the leptotene condition (fig. 2), the parietal cells are dividing or have divided perichinally to form four cells. In most cases no additional divisions occur in these cells. The inner integument is fairly well developed as a collar of meristematic cells around the nucellus approximately at the level of the inner end of the megaspore mother cell. The outer integument is just beginning to appear at the base of the inner integument.

The megaspore mother cell continues to elongate throughout the first reduction division. At diakinesis there are evident fifteen pairs of chromosomes (fig. 3) showing quite a wide range in size. The outer integument is just beginning to close over the base of the inner integument at this time. The chromosomes are regularly arranged at the equatorial plate during the first reduction division (fig. 4). The range in size of the chromosomes is even more evident here. Following completion of nuclear division the cell is divided, usually unequally, by a cell plate. The cell toward the chalazal end of the ovule is considerably larger than the other (fig. 5) and may develop a somewhat thicker wall. The second reduction divisions are also normal, though division of the outer cell may be slightly slower than that of the inner one (fig. 8). By this time the outer integument has enlarged so that it extends forward to about halfway over the inner integument. In most cases the spindles of the second reduction divisions are so arranged that their long axes coincide with the long axis of the original mother cell, and a linear tetrad (fig. 7) is formed. In perhaps one-fourth of the ovules, however, the spindle in the cell nearer the

micropyle is at right angles to the other and a T-tetrad results (fig. 8). When the outer cell, of the two formed after the first division, divides later than the inner one, the daughter cells produced are the first ones to show signs of degeneration. Usually all three outer cells eventually begin to degenerate and the chalazal megaspore, which up to now has been uniformly granular, becomes somewhat vacuolate (fig. 9). Rarely both the inner megaspores may germinate to produce two embryo sacs which may be superimposed at maturity or arranged side by side.

The reduction divisions in the megaspore mother cells occur in the older flowers of racemes which are just emerging from the ground. The first and second reduction divisions in the microspore mother cells occur while the megaspore mother cells are in the early prophase of the first reduction division. The first and second nuclear divisions in the megaspore may be found in the older flowers of racemes of which the flower stalk alone is approximately two to three inches high.

The small vacuoles which appear in the megaspore as it enlarges soon merge and usually form a single large vacuole in the micropylar end of the cell, while the nucleus moves nearer the chalazal end. At times, however, a second and smaller vacuole may be formed in the chalazal end of the megaspore (fig. 10). The degenerating megaspores are much flattened and densely staining at this time. The inner integument now completely encloses the nucellus, and the outer integument encloses the inner up to approximately the level of the megaspore. The first nuclear division in the megaspore (fig. 11) occurs at approximately the same time as the division of the microspore. The spindle is located near the chalazal end of the cell, but one of the daughter nuclei soon moves through the cytoplasm down one side of the cell to the micropylar end. Thus, at the two-nucleate stage the cell has typically a single large vacuole in the center and a nucleus near either end (fig. 12). These nuclei then divide simultaneously (fig. 13) with apparently no consistency as to orientation of the spindles. The outer integument at this time extends up to about the tip of the nucellus and the degenerating megaspores have practically disappeared. The parietal cells start disintegration at about the time of the first nuclear division in the megaspore and at this stage have also practically disappeared. Fagerlind (1941) reported the disintegration of the parietal cells as occurring somewhat earlier in *C. quamash*. With the disappearance of the three outer megaspores and the parietal cells, the developing megagametophyte becomes sub-epidermal.

Following the completion of the second nuclear division, some of the spindle fibers persist as very delicate strands between the daughter nuclei (fig. 14). Fibers which remain in the cytoplasm after completion of the next to last nuclear division in the megagametophyte have been described in *Lilium* by Cooper (1935) and also in other species by various workers. These persistent fibers later bring about

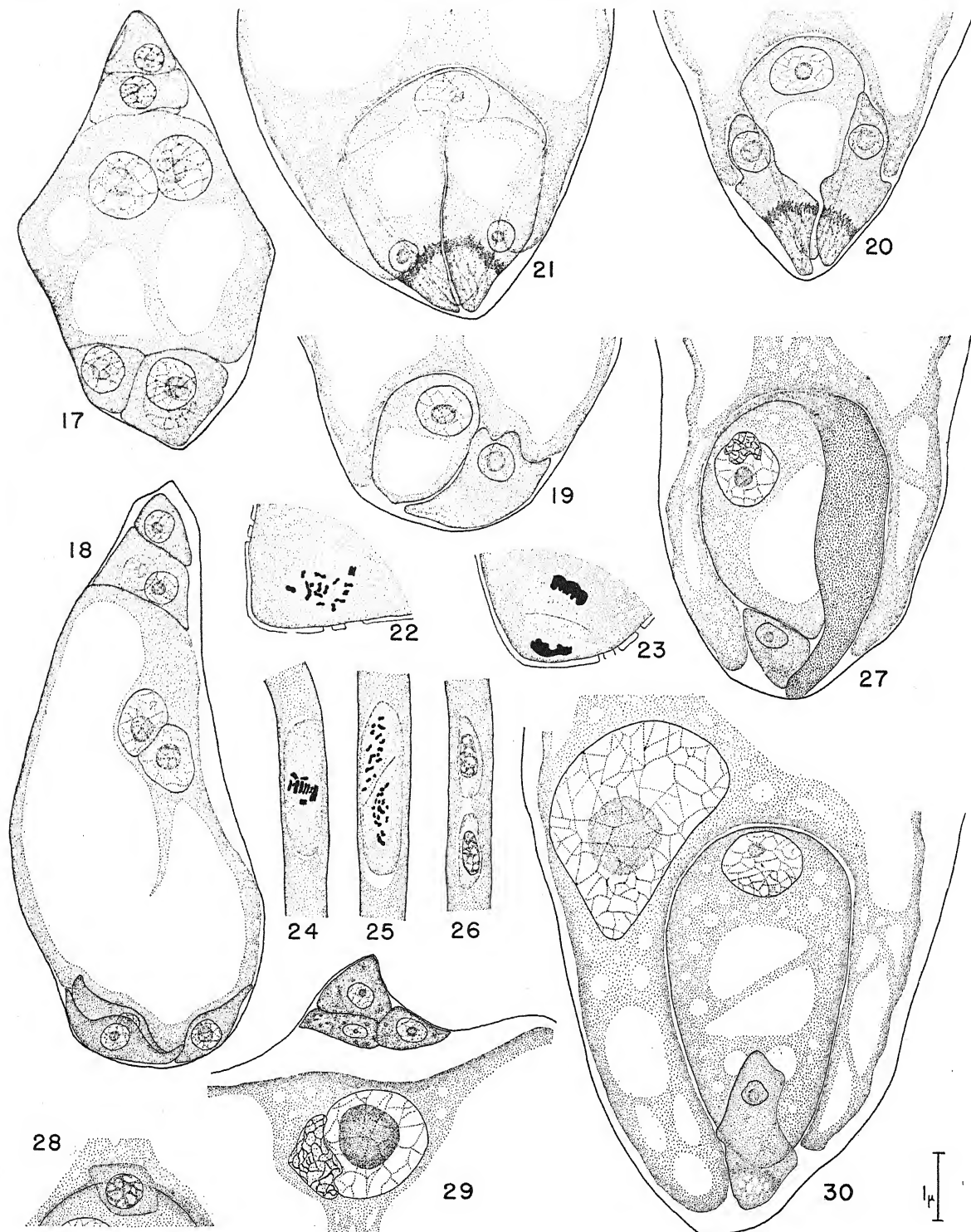


Fig. 17-30. Older megagametophyte, gametogenesis and fertilization.—Fig. 17. Early seven-celled megagametophyte.—Fig. 18. Megagametophyte at time of fusion of polar nuclei.—Fig. 19-21. Development of egg apparatus.—Fig. 22-23. Pre-metaphase and telophase of division in microspore.—Fig. 24-25. Division of generative cell in pollen tube.—Fig. 26. Male cells in pollen tube just outside ovule.—Fig. 27. Pollen tube in one synergid, a portion of the uninjured synergid, and male nucleus fusing with egg nucleus.—Fig. 28. Male cell from secondary pollen tube on surface of egg.—Fig. 29. Chalazal end of gametophyte at time of union of male nucleus and fusion nucleus.—Fig. 30. Zygote enclosed by primary endosperm cell, primary endosperm nucleus and disintegrating synergid.

cell plate formation to aid in the production of the seven-celled megagametophyte.

The ovules in a single ovary, except for a few at the end nearest the style, develop at approximately the same rate up to the four-nucleate stage. There may then follow a period of growth, variable in extent, before the nuclei divide to form the eight-nucleate megagametophyte. Because of this variation there is also considerable variation in the time of formation and in the size of the seven-celled gametophyte. It may be larger or smaller than the average, as its size depends on the length of the growth period during the four-nucleate condition. In general, however, the older flowers of racemes with the flower stalk approximately six inches high will give mostly stages from the early four-nucleate condition to early seven-celled megagametophytes. These older flowers are about 12 mm. long. A complete range of stages may be found at times in a single ovary.

During the third nuclear division in the megagametophyte (fig. 16), the fibers persisting from the second division are present but they are quite delicate and rather difficult to demonstrate. There seems to be no single arrangement of the spindles at the chalazal end of the cell that can be considered as typical. The spindle nearest the chalazal end is usually arranged approximately parallel with the long axis of the megagametophyte, but the other spindle may be oriented in any direction. At the micropylar end the two spindles are usually at approximately right angles to each other, in either of the positions shown in figures 15 and 16. Cell division is accomplished by cell plate formation essentially as in *Lilium* (Cooper, 1935). Cell plates are first formed across the spindles of the third nuclear division (fig. 15). Additional fibers appear in the region of the persistent fibers from the second divisions, and these soon also form typical cell plates. Once initiated, cell division is apparently completed in a short time.

Shortly after cell division is completed, the polar nucleus from the micropylar end of the primary endosperm cell moves up to the vicinity of the chalazal polar nucleus (fig. 17). At the same time there seems to be some increase in the amount of cytoplasm in this cell. A large strand of dense cytoplasm appears which extends from the vicinity of the immature egg apparatus to the polar nuclei. This strand usually passes directly through the large central vacuole, but it may also appear as a dense ridge of cytoplasm along one wall of the cell. The cells at the ends of the young gametophyte are regular in form and are frequently isodiametric or approximately so.

There then follows a rapid enlargement of the primary endosperm cell. The antipodal cells become denser and somewhat reduced in size. The three micropylar cells also become slightly denser and appear to be flattened by pressure exerted by the enlarging endosperm cell (fig. 18). The polar nuclei usually fuse at about this time. Though in some instances the fusion may be delayed for some time, in

every case observed the polar nuclei fuse before fertilization occurs. There is little change in the appearance of the polar nuclei during fusion. The reticulum in each nucleus remains as fine threads and stains lightly throughout the process. The nuclei become flattened on the sides of contact (fig. 18); the nuclear membranes disappear in this region; and the resultant nucleus becomes rounded. The nucleoli also appear to fuse and the fusion nucleus always has a single very large nucleolus (fig. 29). Numerous stages showing this enlargement of the primary endosperm cell and the fusion of the polar nuclei were obtained from the older flowers of racemes with flower stalks approximately sixteen inches high. These flowers were about 22–24 mm. in length. The flower stalks and flowers develop rapidly at this time. There was an interval of only three days between these collections and those made when the flower stalks were only six to seven inches high.

After the primary endosperm cell has enlarged to almost mature size, the egg apparatus begins to grow. The egg enlarges more rapidly than the synergids. The egg nucleus is always in the upper portion of the egg and a large vacuole develops in the lower portion (fig. 19). The synergids also enlarge at one end to form small, cone-shaped cells, each with a projection from the enlarged upper end surrounded by a circular depression (fig. 19). As these cells continue to enlarge, they push into space originally occupied by the primary endosperm cell which, at the same time, apparently sends folds downward to enclose partially both the egg and the synergids. The reticulum of each synergid nucleus begins to lose its chromaticity and becomes rather indistinct. Differentiation of the filiform apparatus begins at about this time and apparently results from the formation of elongate vacuoles in the micropylar end of the cell. The cell wall covering this end of the synergid also stains lightly with safranin at this time. The small projection from the center of the upper end of the synergid then enlarges; the nucleus moves up into this projection; and vacuoles appear in the cytoplasm above the nucleus (fig. 20). The egg is still growing more rapidly than the synergids. Enlargement is due primarily to the increase in size of its single large vacuole. Figure 20 represents a section made at approximately right angles to the plane of figure 19 so that taken together the figures show the relative positions of the egg, synergids and enclosing folds of the primary endosperm cell. The filiform apparatus becomes more distinct and the cell wall at the upper limits of the filiform apparatus begins to appear granular and to stain more heavily with safranin. The granular material in the cell wall forms a complete circle around each synergid at the level indicated. A small ledge is still evident at this time at about the middle of each synergid against which the folds of the primary endosperm cell lie rather closely.

The vacuoles in the upper end of each of the synergids then coalesce to form a single large vacuole (fig. 21), and the synergids enlarge rapidly to mature

size. At maturity the ledges seen at the middle of each synergid in figure 20 are no longer evident. At the upper ends of the synergids the walls are extremely thin and delicate, while along the sides the walls are comparatively heavy. At about the upper limits of the filiform apparatus the walls now stain rather heavily with safranin and particularly so with crystal violet. Figure 21 represents the condition of the micropylar end of the megagametophyte at the time the flower opens.

Up to the four-nucleate or early eight-nucleate stages of the megagametophyte, growth in width occurs mostly at the lower or micropylar end. During the later stages of enlargement of the primary endosperm cell, however, the upper end increases much more in diameter than the lower so that at maturity the megagametophyte is top-shaped. The antipodal cells, already showing signs of degeneration, are crowded into a small chalazal cavity above the center of the primary endosperm cell. Figure 29 illustrates a portion of the chalazal end of the gametophyte at the time of fusion of a male nucleus with the fused polar nuclei. With the exception of the presence of the male nucleus, this figure indicates the approximate condition of the antipodal cells and the fusion nucleus at the time the flower opens. During the final stages of development, the strand of dense cytoplasm extending from the micropylar end of the endosperm cell to the fusion nucleus may increase in thickness, while at the same time there is an apparent decrease in the thickness of the layer cytoplasm around the sides of the cell.

THE MALE GAMETOPHYTE.—Microsporogenesis is typical in all respects and is not illustrated. Both first and second reduction divisions in the microspore mother cell occur while the megaspore mother cell is in the early prophase of the first reduction division. After cell division is completed, there follows a long period in which each microspore increases in size and the spore walls are formed. During this period a large vacuole is present surrounded by a relatively small amount of cytoplasm. The young microspores are not well preserved with Navashin's solution. As the microspore nucleus prepares to divide, however, there is a considerable increase in the amount of contained cytoplasm, and the vacuole becomes much smaller. At this time the microspore and all stages of nuclear and cell division are well fixed with Navashin's solution. While there is some variation in the time of division of various microspores, even in the same anther sac, in general the microspore divides at approximately the time of the division of the megaspore nucleus; that is, in the older flowers when the flower stalk is about two to three inches high. The spindle is oriented with one pole directed toward the spore wall (fig. 22). No irregularities during the division were observed, and the generative cell is cut off by cell plate formation (fig. 28). The generative cell is discus-shaped at first, but it eventually rounds up and becomes enclosed by the tube cell. Elongation of the generative cell does not occur until it enters the pollen tube.

As the pollen tube is formed, the tube nucleus usually moves out of the pollen grain first. The generative cell of necessity becomes cylindrical with rounded ends as it enters the pollen tube. The generative nucleus may already be in the early prophase of mitosis at this time. The process of division apparently takes considerable time and various stages in the process may be found in longitudinal sections of styles taken five to fifteen hours after pollination. During the late prophase the small chromosomes are well spread out over the nucleus and may be counted rather easily. A normal spindle with rather sharp poles is formed (fig. 24) with the equatorial plate extending either practically straight across the cell or at a slight angle. The chromosomes, particularly the larger ones, may be arranged parallel with the spindle. The chromosomes mass closely together during the anaphases, but in the early telophases they soon become dispersed and persist for some time as definite units (fig. 25) before forming a very coarse reticulum. A cell plate appears which results in the division of the generative cell into the two male gametes. Delimitation of the male cells by the formation of vacuoles on either side of the cell plate, as described by Johnston (1941) was not observed, but no special attention was given this phase of the process. The reticulum of the nucleus in each male cell, in contrast with that of the tube nucleus, stains heavily with safranin throughout the passage of the gametes through the pollen tube to the ovule. Nucleoli are not evident in the gametes at this time. While in the style, the male gametes are usually some distance behind the tube nucleus, which remains at the tip of the pollen tube. As the pollen tube enters the micropyle, however, the male gametes are near the tube nucleus. Just outside the ovule the male gametes may be easily observed as distinct cells (fig. 26). As reported by Leffingwell (1930) for *C. quamash*, it requires approximately eighteen hours for the pollen tubes to reach the ovules. This will vary somewhat, of course, with variations in temperature. Covering the placentae and extending to the micropylar tips of all ovules at maturity, there is a mass of apparently gelatinous material. The pollen tubes grow in or on this material but are not restricted to it. The material does not stain when the safranin-aniline blue technique is used, but it stains heavily with fast green.

FERTILIZATION.—Many stages in double fertilization were found in material collected twenty to twenty-five hours after pollination. As pointed out by Wylie (1941) for *Vallisneria*, apparently only a very short interval elapses here between the emergence of the gametes from a primary pollen tube and fertilization. The only complete male gametes observed in the megagametophyte in this study were from secondary pollen tubes. Such gametes were observed to have become more rounded after their release from the pollen tube (fig. 28). There is a very delicate cell membrane and the nucleus itself is almost spherical. The reticulum of the male gamete

in the pollen tube outside the ovule stains heavily with safranin, but upon reaching the megagametophyte, from the time of emergence from the pollen tube to the completion of fertilization, the reticulum stains only lightly with safranin but rather heavily with aniline blue.

As the pollen tube enters the megagametophyte, usually only one synergid is penetrated. This one is filled completely by the pollen tube, and the combined cells stain rather heavily with safranin. The thin membrane across the top of the synergid is ruptured and contents from the pollen tube, and probably some from the ruptured synergid, flow out across the top of the egg (fig. 27). In the earliest stage of fertilization observed, the rounded male nucleus was already inside the egg in contact with the egg nucleus. A nucleolus is not yet evident in the male nucleus at this time. The male nucleus then becomes somewhat flattened, and small lobes appear to reach out over the surface of the egg nucleus (fig. 27). Following the dissolution of the intervening membranes the two nuclei are incorporated into a single nucleus. However, the reticulum of the male nucleus is evident for some time in the zygote because of its coarser nature and greater chromaticity. This reticulum develops a small nucleolus after it is incorporated in the zygote nucleus (fig. 30). This late development of the nucleolus of the male gamete has been described by Gerassimova (1933) and others.

The second male nucleus is apparently released from its cytoplasm as the gamete enters the primary endosperm cell or very shortly thereafter. It immediately moves up the large strand of cytoplasm leading to the fused polar nuclei. It reaches the fusion nucleus only a very short time after the other male nucleus has begun to fuse with the egg nucleus. The stages in nuclear fusion are approximately the same as those in the egg except that the male nucleus usually enlarges to a somewhat greater extent before being incorporated into the primary endosperm nucleus (fig. 29). The fate of the tube nucleus and of the nucleus of the destroyed synergid was not determined.

During this time the folds of the primary endosperm cell continue to enlarge and enclose the egg apparatus more completely. After fertilization is completed, the destroyed synergid and the pollen tube lose their chromaticity and are rapidly absorbed. The uninjured synergid decreases in size, rapidly at first, because of the passage of material from the vacuole at the upper end (fig. 30). It soon disappears completely. There is a rapid accumulation of cytoplasm in the zygote, and the primary endosperm nucleus moves down near the zygote before it begins to divide. By this time the folds of the primary endosperm cell are much enlarged and almost completely enclose the zygote (fig. 30). The antipodal cells have degenerated to a considerable extent by this time and soon become entirely absorbed. Division of the primary endosperm nucleus then occurs, followed by several successive nuclear

divisions before the zygote divides. These early divisions in the endosperm occur approximately at the time when the perianth segments begin to dry and become twisted around the ovary.

SUMMARY

There are fifteen pairs of chromosomes in *Camassia Leichtlinii*. The megagametophyte follows the normal-type of development. Cell division is accomplished by cell plate formation by fibers of both the second and third nuclear divisions in the formation of the gametophyte. The polar nuclei fuse before the gametophyte is mature. The synergids are first flattened by pressure from the enlarging primary endosperm cell. They then enlarge and develop into cone-shaped cells showing a filiform apparatus in the cytoplasm. A dark-staining ring of granular material in the cell wall appears at the upper level of the filiform apparatus. The three antipodal cells degenerate soon after fertilization.

The pollen grains are two-celled. The generative cell in the pollen grain is round and enclosed by a distinct membrane. It becomes cylindrical as it enters the pollen tube and, following a typical mitosis, is divided by the formation of a cell plate. The male gametes persist as complete cells until they are discharged into the megagametophyte.

Various stages in the development of the gametophytes were correlated with the external development of the flower stalk and flowers.

The pollen tubes reach the ovules approximately eighteen hours after pollination, and fertilization occurs two to five hours later. Only one synergid is penetrated by the pollen tube as it enters the megagametophyte. Secondary pollen tubes were also observed. Double fertilization occurs at approximately the same time in a given megagametophyte. A nucleolus is not developed by the reticulum of the male gamete until nuclear fusion is completed.

It is recommended that *Camassia* be used in the classroom instead of *Lilium* to demonstrate development of the Angiosperm megagametophyte.

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SPIRAL GROWTH AND REVERSAL OF SPIRALING IN PHYCOMYCES, AND THEIR BEARING ON PRIMARY WALL STRUCTURE¹

Edward S. Castle

MOST INVESTIGATORS would agree that there is a high degree of orientation in the structure of the plant cell wall. In some measure this extends even to the structure of the tenuous, growing, primary wall of the enlarging cell. Such structural orientation is shown largely by the occurrence of cellulose in the form of microfibrils, themselves crystalline, lying in one or more preferred orientations, usually in the plane of the cell surface. Cell enlargement is accompanied by expansion or growth of the primary wall, and involves the deposition of new wall material in an oriented pattern.

What determines the characteristic form of cell growth and the pattern of its wall structure has never been clear. Plant growth hormones probably act not directly on the wall but at some prior stage in the complex process of growth. On the other hand, microscopic and X-ray investigations have been most successful in dealing with secondary wall structures where the cell is already beyond the stage of active enlargement. Thus, although the primary wall is of the greatest physiological interest, we have the least satisfactory knowledge of its fine structure and mode of growth.

There is, however, an additional line of evidence that can be brought to bear on primary wall structure. In some types of cell the orientation of the growth process can be studied. Where this has been possible, growth has been found to be highly oriented, and it appears that such growth co-exists with oriented structure of the growing wall. Oriented growth may be defined as active enlargement along a preferred axis or axes. For example, many cylindrical cells have attained their shape primarily by elongation, and might as a first approximation be said to possess growth oriented along their long axis. Tissue cells, however, may have been passively oriented in their growth by mechanical influences. Hence, it is not possible to consider from this point of view such interesting cell types as the cambial elements or the much-studied cells of the *Avena* coleoptile.

The occurrence of oriented growth in cells which are at all free to determine their own shapes clearly indicates the existence of a directional property in the structure of the growing wall. Some authors have regarded the preferred axis of cell enlargement as directly determined by the existing wall structure (Bonner, 1935; van Iterson, 1937; Diehl, *et al.*, 1939; Ziegenspek, 1939, 1941). Broadly interpreted, this view should permit inferences as to orientation of structure from studies of oriented primary wall growth.

It is regrettable that we lack adequate information about the orientation of growth in cells whose primary wall structure is best known, such as the

cotton fiber (Anderson and Kerr, 1938; Berkley, 1939; Hock, Ramsay, and Harris, 1941). Almost the only available data relate to the growth of the sporangiophore of *Phycomyces*, where a spirally oriented form of growth occurs. The mature cylindrical sporangiophore elongates by growth which is restricted to an apical zone proximal to the terminal sporangium. The axis along which elongation takes place is not parallel to the long axis of the cell, but makes an angle with it which may be as much as 30°. Hence, the sporangium and the terminal region of the cell are rotated about the long axis during growth, and the cylindrical sporangiophore forms a spirally-wound wall structure (Oort, 1931; Oort and Roelofsen, 1932; Castle, 1937, 1938).

Spiral growth is by no means confined to *Phycomyces*. It exists in the related fungus *Mucor mucedo*,² and according to Astbury and Preston (1940) occurs in *Cladophora* where, significantly, spiral wall structure also is found. It is likely that suitable growth studies will reveal that the incidence of spiral growth is more general than has been supposed. Heyn (1939) has listed some five attempted explanations of spiral growth, as many as two "theories" being ascribed to the present writer. It is evident that where there is so much theory there must be a dearth of fact, and the work described in this paper was undertaken primarily to give a more complete picture of the facts of spiral growth in *Phycomyces*.

MATERIAL AND METHODS.—The "plus" strain of *Phycomyces blakesleeana* was grown in small vials on autoclaved white flour paste, which supports luxuriant growth. Isolated sporangiophores were obtained by clipping away all but one of those present in a culture. In order that growth might be followed in localized regions, individual markers were attached to the sporangium or to the sporangiophore with a micromanipulator. Single spores of *Lycopodium* were found to make excellent markers. They were easily attached by a touch of the micromanipulator needle and showed up well in photographic records of growth.

In general, automatic photographic recording was employed, using a special camera magnifying ten times. The culture vessel with a suitably marked sporangiophore rested in a moist, glass-walled chamber with a constant dim light overhead to ensure vertical growth. The camera was placed in front of the chamber and was driven parallel to the chamber at a slow, constant rate by a lead screw turned by a synchronous motor acting through a train of gears. Periodically, the sporangiophore was brightly illuminated with red light from one side and automatically photographed, the travel of the camera being briefly in-

² I am indebted to Dr. Kenneth B. Raper of the Northern Regional Research Laboratory for cultures of *M. mucedo*.

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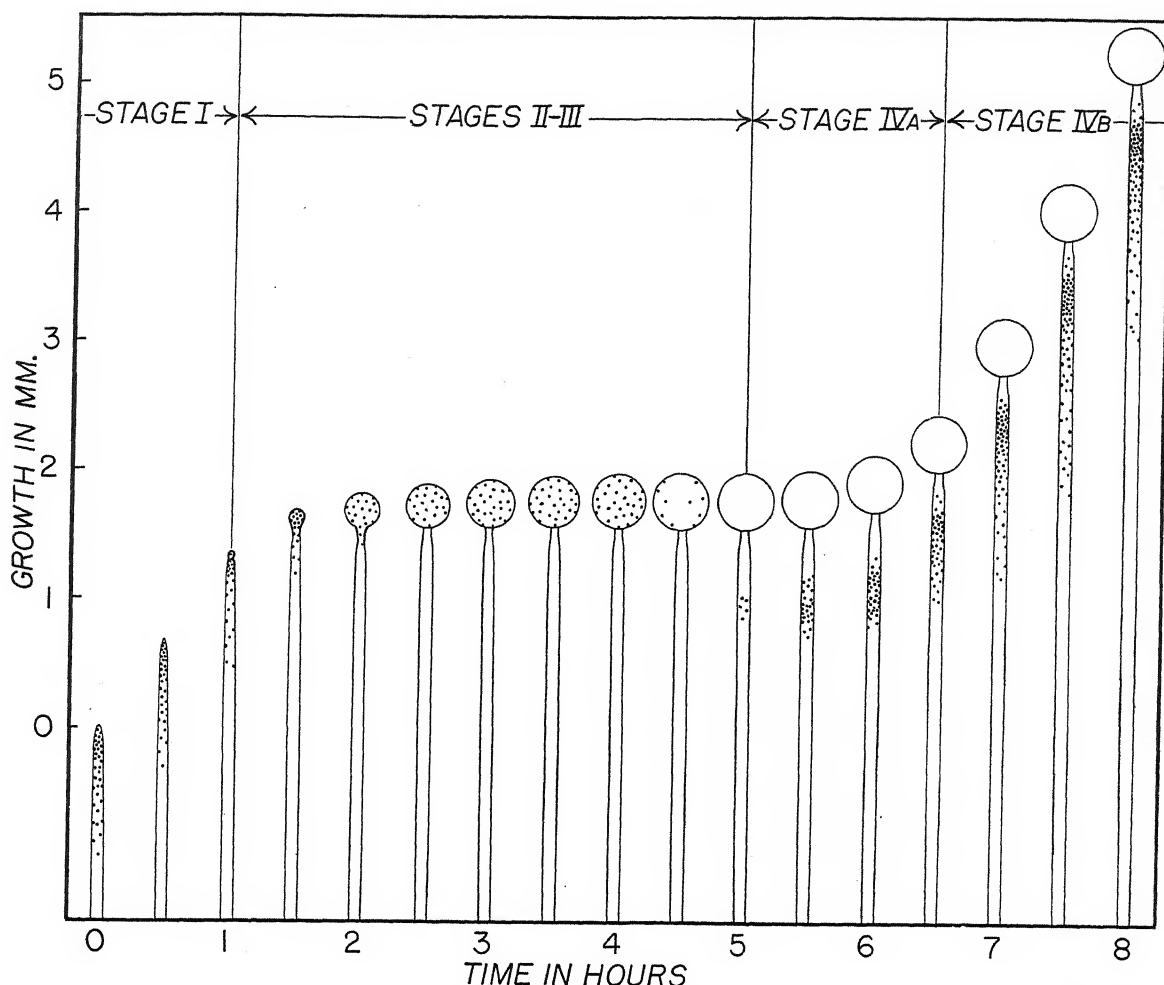


Fig. 1. Diagram of developmental stages of the sporangiophore of *Phycomyces*. Regions in which growth is taking place are stippled. Note apical growth of tubular sporangiophore in stage 1. When the sporangium appears as a terminal swelling, growth in length shortly ceases, and growth during stage 2 becomes confined to sporangial enlargement. In stage 4a elongation sets in again, and the sub-sporangial growing zone lengthens as the growth rate accelerates. The rotary component of spiral growth is not shown in these diagrams. In stage 1 the axis of growth is directed sinistrally, in stages 2-3 growth is unoriented, in stage 4a dextral spiraling occurs, and in stage 4b sinistral spiraling takes place (see text).

interrupted during the exposure. The red light was outside of the phototropic range, and was cooled by passage through a water filter. Because the object was brightly illuminated against a dark background, a series of exposures could be obtained on a single plate. Figures 2, 3 and 4 are contact prints of such records, slightly reduced in reproduction. A modified form of the same apparatus permitted taking serial pictures of a cell from above instead of from the side, although not automatically. Figures 6 and 7 are enlargements of such records, and constitute the most direct way of demonstrating the rotary component of growth.

STAGES OF GROWTH.—Four developmental stages in the growth of the sporangiophore of *Phycomyces* were described by Errera (1884). In stage 1 the sporangiophore grows upward from the mycelium

as a simple pointed tube with apical growth; in stage 2 the tip of the cell begins to swell to form the sporangium, and elongation ceases; stage 3 is a period of two to three hours during which no further swelling of the sporangium occurs and elongation is at a standstill; in stage 4 elongation begins again and continues for many hours. During this last stage the previously yellow sporangium rapidly becomes brown and then black in color as the spores ripen. This is the "grand period" of growth which has been widely used for growth and tropistic studies.

Graser (1919) was unable clearly to separate stages 2 and 3, but Grehn (1932) believed the distinction to be valid. In the present paper stages will be designated as by Errera, although 2 and 3 will for convenience be treated together. If stage 3 is a

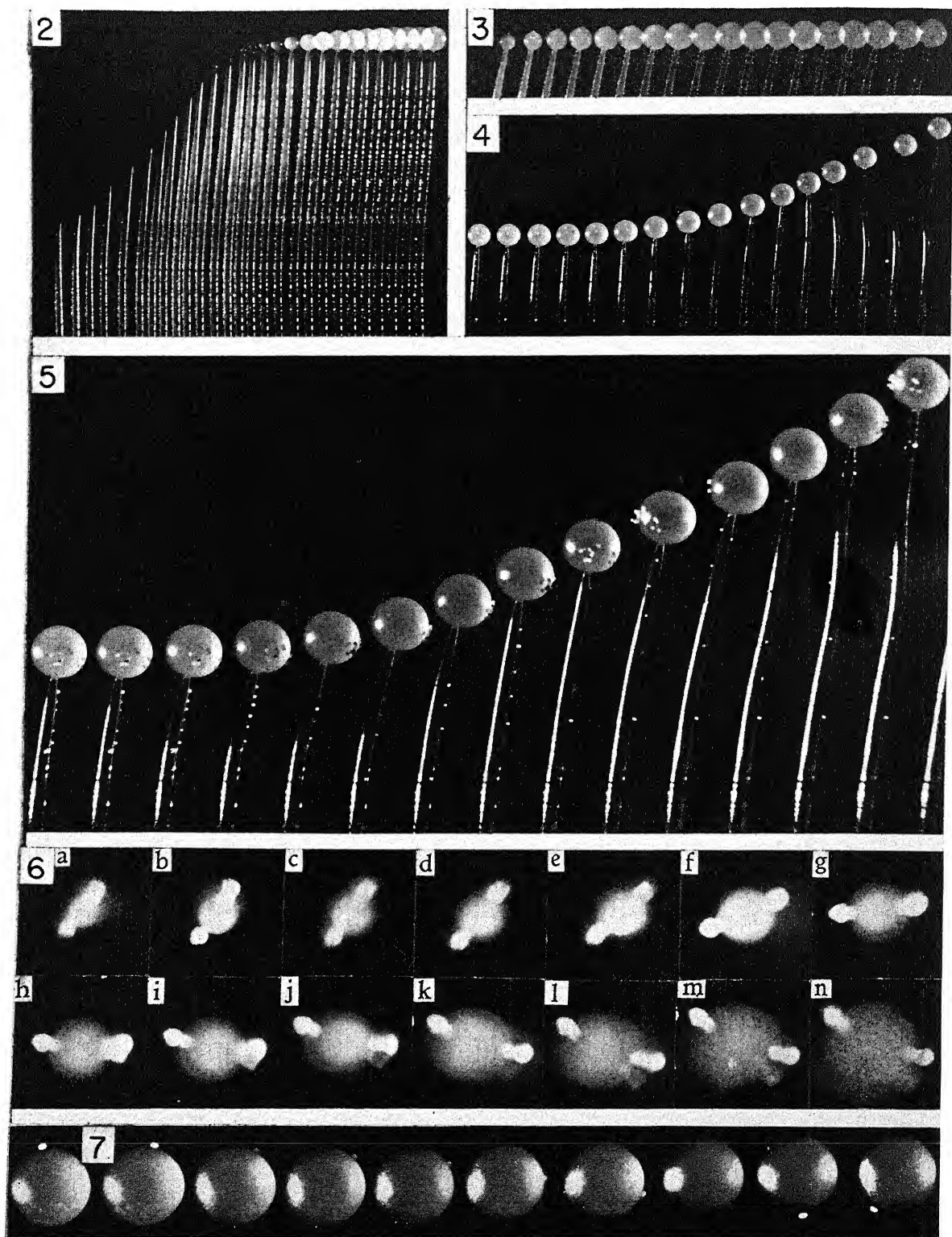


Fig. 2-7. Photographic records of growth.—Fig. 2. Growth of sporangiophore and process of sporangium formation, stages 1 and 2. Note characteristic refractive droplets exuded by stage 1 sporangiophore. Time lapse interval fifteen minutes. $\times 9$.—Fig. 3. Swelling of sporangium, stage 2, with markers attached to pole of sporangium. Rotation absent. Time lapse interval fifteen minutes. $\times 9$.—Fig. 4. Initiation of growth after sporangium formation. Markers attached

separate entity, it is set off from stage 2 only by the complete cessation of sporangial enlargement. As will be seen below (fig. 1), stage 4 can properly be divided into two substages, 4a and 4b, on the basis of their different modes of spiral growth.

RESULTS.—It is convenient to describe the observations on growth in terms of the various stages described above and illustrated in figure 1.

Stage 1.—Figure 2 shows on the left a series of pictures at fifteen-minute intervals of the young, stage 1 sporangiophore. The total height of the cell attained before swelling of the tip begins (stage 2) varies greatly with the culture conditions. In the small vials commonly used, stage 1 cells rarely exceed 3 to 5 cm. in length. Cultured on the same medium in liter flasks, however, the cells usually grow to lengths of 10 cm. or more before sporangium formation begins. In the figure, a marker attached to the left side of the cell not far below the tip appears as a small white spot. In successive pictures this spot can be seen to move upward with decreasing velocity, finally becoming stationary as it passes out of the growth zone. The length of the growth zone is thus given by the distance from the tip of the cell to the marker when it just ceases further upward movement. At this point it will be noted that a number of other white spots appear in the photograph around the marker. These are visible farther down the cell in the earlier pictures, and extend above it in later ones, giving the dotted appearance to the margins of the cell in figure 2. The spots are reflections from droplets formed on the outside of the cell, and are characteristic of stage 1 sporangiophores. They first appear just below the lower limit of the growth zone, and rapidly increase in size. Their presence is an additional indication of the length of the growing zone, which extends from just above the first-formed, uppermost droplets to the tip of the cell. Doubtless these droplets are comparable to those which are exuded so conspicuously by the sporangiophores of *Pilobolus*. It is striking that droplets are never formed on the wall during any of the later stages of growth of *Phycomyces*, not even in stage 4 (cf. fig. 4 and 5).

Although consistent spiral growth of stage 1 cells has not previously been found (Castle, 1936), re-examination shows that these cells do show spiral growth. If pains are taken to attach markers to the extreme tip, they are found to progress slowly around the cell as well as to be carried upward in the usual way by growth occurring below them. There is, therefore, a rotary as well as a longitudinal component of growth. That rotation is not due to passive torsion of the whole cell can be seen by

simultaneous observation of markers attached below the growth zone.

Figure 6, *a* to *n*, shows photographs taken of a stage 1 cell from above, with markers attached on opposite sides of the growing tip. In these pictures the cell grows directly toward the camera, and in successive pictures taken at fifteen-minute intervals the markers can be seen to move clockwise around the cell's long axis. (This axis is perpendicular to the plane of the paper.) Of the fifteen stage 1 cells tested, nearly all showed this rotary component of growth, though not all to the same degree. Only two of the fifteen seemed to have no rotary component. Rotation in this direction corresponds to growth occurring along a steep left-hand spiral which runs around the cell like the thread on a left-handed screw.

Even when markers are attached at the extreme tip of the cell, the maximum rotation which has been observed is about 90° (cf. fig. 6 *a*, 6 *n*). By contrast, markers attached in the growth zone of stage 4 cells often make one and one-half to two complete revolutions about the axis (Castle, 1937). Thus in stage 1 markers pass out of the growth zone relatively fast, due in part to the fact that the growth zone is on the average only about half as long in stage 1 (1 mm.) as in stage 4 (1.5 to 2 mm.). This fact has made it difficult to determine the angle of inclination of the spiral, but in a few favorable cases it can be roughly estimated as about 10° . This angle represents the deviation of the spiral axis of growth from the long axis of the cell. It is noteworthy that qualitatively the direction of the spiral is left-handed (sinistral) and in this respect similar to the characteristic sinistral growth in stage 4.

Stages 2 and 3.—Stage 2 begins with the swelling of the tip of the sporangiophore and continues through maturation of the sporangium. As the tip swells, elongation below it falls off and finally ceases, so that all growth becomes confined to sporangial enlargement. During this enlargement a mass of yellow, carotenoid-rich protoplasm passes up the sporangiophore and into the sporangium, and the initially hyaline swelling becomes brightly pigmented. The taking over of growth by the sporangium is diagrammed in figure 1, and shown in actual records in figures 2 and 3.

Attachment of markers to the sporangium shows that there is no rotary component of growth during sporangial enlargement. The markers on top of the sporangium in figure 3 separate slightly as the circumference of the sporangium increases, but show no trace of rotation. The chief point of interest, then, is the absence of oriented growth during stages 2

to face of sporangium and of sporangiophore. Rotation begins as dextral, then reverses and becomes sinistral. Time lapse interval fifteen minutes. $\times 9$.—Fig. 5. Stages 4a and 4b as in preceding figure. Markers on face of sporangium and on side of sporangiophore. Note initial dextral rotation followed by reversal. Time lapse interval fifteen minutes. $\times 19$.—Fig. 6. Stage 1 sporangiophore photographed from above, with markers attached on opposite sides of tip. Growth is toward the camera. Clockwise rotation of markers indicates sinistral spiral growth. Markers separate as growing apex increases in diameter. Time lapse interval ten minutes. $\times 75$.—Fig. 7. Stage 4b sporangium photographed from above, with markers on equator of sporangium. Growth is toward the camera. Rotation is clockwise, indicating sinistral spiral growth. Time lapse interval five minutes. $\times 23$. The stationary white spot on the left side of each sporangium in figures 3, 4, 5 and 7 is a highlight, and remains in a fixed position in spite of rotation.

and 3, although they are both preceded and followed by stages having oriented growth. As mentioned above, stage 3 appears to be only a brief prolongation of stage 2 in which sporangial enlargement has ceased. It is difficult to determine whether this is really a separate stage because the increments in diameter of the sporangium have by now become small.

Stage 4.—With the sporangium completed, elongation of the sporangiophore again commences. Since this is the stage which exhibits conspicuous spiral growth, the initial period of stage 4 is of especial interest.

It was found that at the outset of renewed elongation and for a period of an hour or more, growth is again spirally oriented, but is *dextral* (right-handed) instead of sinistral. At the end of this time, the direction of rotation reverses and becomes sinistral. Normal sinistral spiral growth then remains in force for the duration of stage 4, a matter of many hours or days. This transitory period of reversed, dextral spiraling was found uniformly in the large number of cells examined, and is evidently a normal phase of growth.³ It has, therefore, been designated stage 4a to distinguish it from the sinistral phase which follows, the latter being termed stage 4b (fig. 1). The separation of these two sub-stages is based on the hitherto undetected reversal of the direction of spiraling. Throughout stage 4a and well into stage 4b the velocity of simple elongation is smoothly accelerating and appears unaffected by the reversal of the rotary component of growth (fig. 8).

Reversal is clearly shown in figures 4 and 5. Markers have been attached to the sporangia, and appear as irregularly spaced white (or dark) spots on the nearly spherical sporangia. The large white equatorial spot which appears on the left side of each sporangium is not a marker but a highlight, which naturally remains in a fixed position in spite of rotation of the sporangium.

Beginning with the first picture on the left in figure 5 and progressing to the right, the markers can be seen in the first six pictures apparently to move across the face of the sporangium until they appear silhouetted on its right margin. This motion is actually due to dextral spiral growth of the sporangiophore, stage 4a. In the eighth picture from the left,

³ It was present in cells grown at 14° as well as at 22°–25°C.

the markers have started back across the face of the sporangium, and they continue in this direction until in the twelfth picture they have disappeared on the other side. In the next picture they reappear on the right margin, and henceforth this right-to-left travel across the face of the sporangium will continue. This is the normal sinistral spiral growth of the sporangiophore, stage 4b (fig. 7).

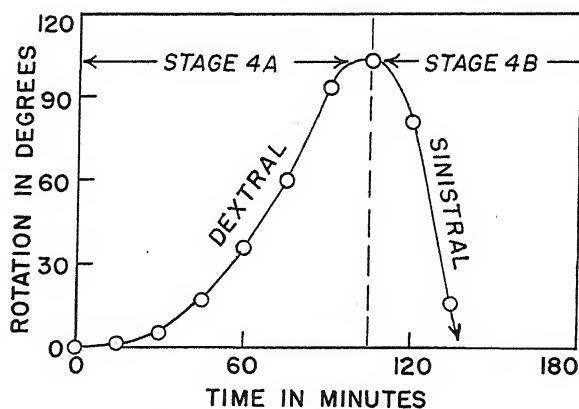
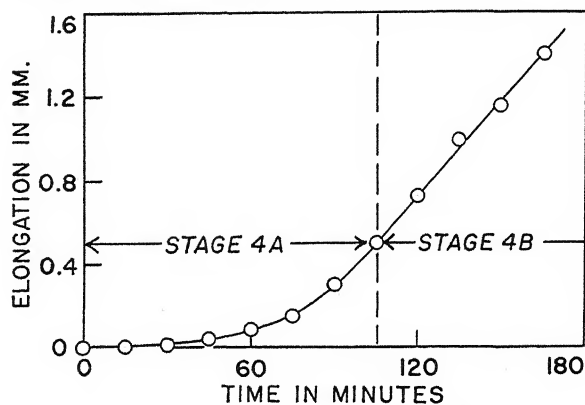


Fig. 8-9.—Fig. 8 (above). Time course of elongation after sporangium formation. At the dotted vertical line dextral spiral growth reverses and becomes sinistral (see fig. 9).—Fig. 9 (below). Time course of rotation after sporangium formation, showing reversal of direction of spiraling.

TABLE 1. Elongation and rotation in stage 4a, with calculated initial angles of dextral spiraling in stage 4a and of sinistral spiraling in stage 4b.

Cell No.	Duration of stage 4a in hours	Elongation in stage 4a in mm.	Rotation (dextral) in stage 4a	Initial angle of dextral spiraling in stage 4a	Angle of sinistral spiraling in stage 4b
1	1.25	0.33	53°	11°	8°
2	1.25	0.38	66°	11°	7°
3	1.50	0.40	50°	7°	7°
4	1.75	0.45	84°	10°	4°
5	1.50	0.50	104°	15°	12°

On five separate records comparable to figure 5, measurements were made of the duration of dextral spiral growth and of the magnitude of its two components, elongation and rotation. These are given in the second, third, and fourth columns of table 1. Duration was measured only to the nearest quarter hour. Elongation was derived from direct measurements on the record. Rotation was calculated from changes in the position of the markers on the face of the sporangium. From table 1 it will be seen that the average duration of stage 4a is about an hour and a half at room temperature (22°–25°C.). Elongation during this time is between 0.3 and 0.5 mm., and the concomitant dextral rotation is between 50° and 100°.

Detailed data for cell No. 5 are plotted in figures 8, 9, and 10. Figure 8 shows the time course of elongation for three hours after the start of stage 4a. There is no perceptible change in the growth curve at the point where dextral spiraling reverses and becomes sinistral (vertical broken line). Figure 9 shows the corresponding course of rotation. For nearly an hour and a half the rate of rotation (dextral) is smoothly accelerating, then it begins to fall off and within half an hour sinistral rotation has become established. The further course of the curve in stage 4b will cut the baseline, which is arbitrary, and continue steeply downward, indicating continued sinistral spiraling.

More light is shed on the process of growth if the time factor is eliminated, and the rotational component plotted directly against elongation. If a fixed relation exists between the two components, we expect a linear plot the slope of which is a measure of the angle of spiral growth. The data for cell No. 5 are plotted in this manner in figure 10. Here it is seen that at the outset of stage 4a rotation keeps pace with elongation, as indicated by the linear ascending portion of the curve. This means that dextral spiraling begins at a fixed angle the moment any growth occurs. Regular spiraling at this angle continues for over an hour, then rotation begins to fall behind elongation, ceases where the curve passes through a maximum, and reversed spiraling (sinistral) then commences. The baseline in figure 10 is again arbitrary, and sinistral spiraling will normally continue throughout stage 4b. The transition period from stable dextral to sinistral spiraling, represented by the curved, upper region of the plot in figure 10, lasts about three quarters of an hour. At the end of this time, a stable angle of spiraling has been re-established, but in the opposite direction (sinistral). All five of the analyzed growth records show these same features, with minor differences in times and angles.

From simultaneous measurements of rotation and elongation it is possible to calculate α , the approximate angle of spiral growth, expressed as the deviation of the axis of growth from the long axis of the cell.

$$\tan \alpha = \frac{\pi D \theta}{360 h}$$

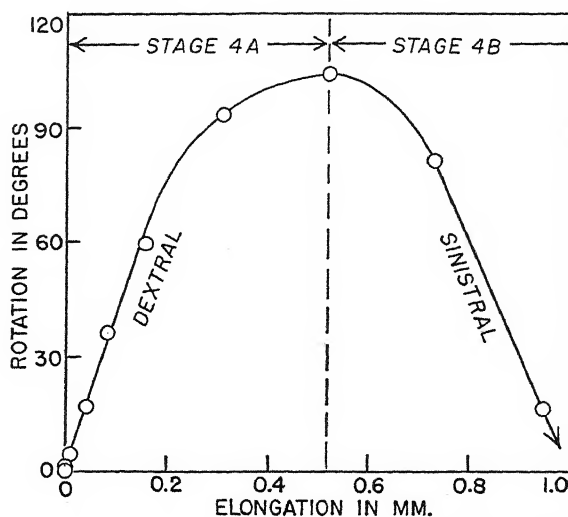


Fig. 10. The two components of spiral growth plotted against each other. At the outset of stage 4a, dextral spiraling begins at a fixed angle as shown by the linear ascending limb of the curve. Reversal occurs at the dotted vertical line, and shortly thereafter a stable angle of sinistral spiral growth is established.

where D = diameter of the cell, θ = rotation in degrees, and h = elongation. The linear slopes in figure 10 give the ratio θ/h , and D is measured directly. Columns 5 and 6 of table 1 give the approximate calculated values of α , both dextral and sinistral, for each of the five cells analyzed. The initial angles of dextral spiraling (column 5) are between 7° and 15°, and fall within the range normally found for sinistral spiraling (Castle, 1936). The ensuing angles of stable sinistral spiraling (column 6) tend to be somewhat smaller than the preceding dextral angles.

A number of cells were studied both at the end of stage 1 and during stage 4a to make sure that the dextral spiraling which appears immediately after sporangium formation was not previously present. All cells so studied showed normal sinistral spiraling at the very end of stage 1. During stages 2 and 3 there was no rotation of any kind, but the same cells exhibited dextral spiraling at the outset of stage 4a.

An important related point concerns the locus of renewed growth when stage 4 commences. During sporangium formation the cell wall below the sporangium shows no growth. It remains, in effect, a proximal portion of the wall of the simple stage 1 sporangiophore. The renewal of growth in stage 4 occurs precisely in this sub-sporangial region of the cell (see stippled regions in fig. 1). When this region was growing previously, its growth axis was directed sinistrally, yet in stage 4a it starts to grow again but with the axis of growth directed dextrally. Something has happened during the formation of the sporangium to cause the same cell wall to reverse its direction of spiral growth, although within about an hour it again returns to the sinistral pattern.

The initiation of growth during the early part of stage 4a was studied in detail by attaching during stage 2 a series of markers along the front or side of the sub-sporangial region which was destined to become the growing zone in the ensuing stage 4 (cf. fig. 5). When growth started, changes in the separation of these markers or in their relative positions to the right or left permitted estimation of the location of growth and of its dextral or sinistral components.

Such studies show that at the outset of stage 4a growth first begins at a point about 0.6 mm. below the sporangium. For a time the growing zone is short, then it progressively lengthens upwards toward the sporangium. Within two hours the growth zone has reached its typical length of 1.5 to 2 mm. During this time the total rate of elongation has also been increasing. While dextral growth takes place, all markers within the growing zone show displacement to the right, indicating that the entire growth zone participates in the dextral growth. As dextral growth lessens toward the end of stage 4a, the markers in the growth zone progressively lessen their dextral motion. When sinistral growth begins, all the markers in the growth zone begin to move sinistrally. From these observations it is clear that the gradual transition from dextral to sinistral spiral growth is not due to the fact that some parts of the growth zone continue to grow dextrally while others begin to grow sinistrally and gradually take over control of the orientation of growth. Rather it is that the entire growth zone contributes to the total dextral component measured at the sporangium, and then the dextral orientation within the whole growing zone gives way to a sinistral orientation within that same growth zone.

Discussion.—Study of the early stages of growth of *Phycomyces* has revealed a remarkable complexity yet consistency in its growth pattern. The chief new aspects of this pattern are as follows:

- (1) Contrary to previous belief, growth of the sporangiophore during stage 1 commonly possesses a rotational component, the axis of growth being directed sinistrally. If such sinistral growth is reflected in a helical wall structure, this has so far escaped detection (Castle, 1938). A critical reexamination of structural orientation in the primary wall of stage 1 cells is, therefore, called for.
- (2) Sporangial enlargement (stage 2) occurs as a spherical inflation of the tip of the sporangiophore. This process possesses no rotational component and appears to be a case of non-oriented growth. The factors which initiate sporangial development and the associated loss of oriented growth are unknown. Growth in this stage resembles passive inflation of an isotropic membrane, as if structural elements in the sporangial wall had no preferred orientation beyond that of lying tangentially in the surface.
- (3) When growth of the sporangiophore is established in stage 4, it immediately shows dextral spiral growth, although previously this same region of the cell had been growing sinistrally. After dextral spiral growth for somewhat more than an hour

(stage 4a), the orientation of growth reverses and becomes sinistral. Thereafter this sinistral mode of growth is maintained (stage 4b).

It would be rash to attempt to explain such an array of facts in terms of any one of the existing theories of spiral growth. Although Heyn (1939) has listed some five "theories" of spiral growth, there are in reality three basic ideas involved. First, the concept that protoplasmic streaming orients chain molecules which are being built into the wall (Pop, 1938). Second, consideration of geometrical factors in the curvature of the growing wall which influence the distribution of elastic stresses and of chain molecules or fibrils bearing these stresses (Castle, 1936). Third, the idea that growth occurs as slippage along inclined planes of weakness in a crystalline grid (Heyn, 1936, 1939). Each of these concepts is too limited alone to permit explanation of the complex nature of spiral growth in *Phycomyces*.

Evidence has accumulated that plant cell walls may consist of a number of superimposed lamellae in which the orientation of microfibrils of cellulose varies from layer to layer (Bailey, 1940; Bailey and Berkley, 1942). In particular, Astbury and Preston (1940) have described for the walls of *Valonia* and *Cladophora* a series of lamellae in which the cellulose chains in any one layer make an angle of nearly 90° with those in the layers next above and below. In the primary wall of cotton, Anderson and Kerr (1938) have shown three systems of microfibrils in a single wall thickness: two opposing systems of crossed, spirally-wound fibrils, and a third system transverse to the long axis of the cotton fiber. These findings have been confirmed by Hock, Ramsay, and Harris (1941). Preston, long an advocate of a single spiral orientation in conifer tracheids (1934, 1939a), has recently stressed the significance of the double crossed spiral pattern such as is found in *Valonia* and *Cladophora* (1939b).

Should the crossed spiral arrangement turn out to be widespread in primary walls, it would be of importance in the interpretation of growth. There is evidence that elongation of plant cells tends to take place perpendicular to the direction in which the cellulose chains principally run, if only because separation of the chains and hence growth is possible in that direction. Since this fact admits a relation between primary wall structure and growth, it can be concluded that spiral growth reflects the occurrence of spirally oriented wall structure.

If a primary wall were composed of only two spiral systems which cross, we could not foresee whether growth would occur in the direction perpendicular to one set of fibrils or to the other, or whether growth might not take place along some intermediate resultant path of least resistance. Factors governing the outcome in a cylindrical cell would certainly be: (1) the angle of inclination of each spiral system from the long axis of the cell. These angles need not be the same, as in *Cladophora* where one system is a steep, the other a low, spiral. It is interesting that Astbury and Preston report clear

evidence of spiral growth in *Cladophora*, although no measurements of its inclination were made. (2) The angle of crossing of the two systems. In *Valonia* and *Cladophora* this angle approaches 90° . In cotton it is 40° or less. (3) The relative strengths of the opposed spiral systems.

It is speculative at the moment to suggest that the reversals of spiraling which occur in *Phycomyces* are founded upon a system of crossed spirals in the primary wall. Yet the manner in which the angle of spiral growth shifts from a stable dextral to a stable sinistral angle is hard to visualize in any other terms. The facts show that in stage 1 spiral growth tends to occur along an axis inclined sinistrally from the long axis of the cell. This might indicate a structural system the predominant orientation of which is along a dextral spiral of low pitch. While the cell "rests" during sporangium formation, a sinistrally oriented structural system may be added to the wall, so that when growth begins in stage 4a its spiral component is predominantly directed perpendicular to this new structural system, i.e., dextrally. Why spiral growth shortly reverts to the fundamental stable sinistral pattern is, however, not clear.

Study of the orientation of growth brings important evidence to bear on the structure of the growing wall. Except in the cases cited above where definite crossed spiral systems have been described, primary wall structure has been variously pictured as amorphous, statistically isotropic, or at best a confused tangle of cellulose microfibrils. The complex but consistent sequence of changes in the orientation of growth of *Phycomyces* clearly shows that some-

thing imposes definite directional properties upon the process of growth. It appears most plausible that these directional properties are determined by the structure of the wall itself. Whether they are explicable in terms of oriented fibrillar systems or of as yet unknown factors remains for a closely coordinated study of growth and of structure to show.

SUMMARY

Largely by automatic photographic recording, a study was made of the rotational and elongational components of growth of the sporangiophore of *Phycomyces*. The presence of a rotational component indicates the occurrence of spirally oriented growth. Prior to sporangium formation, the simple tubular sporangiophore commonly exhibits spiral growth which is directed *sinistrally*. During swelling of the cell tip to form the sporangium, there is no rotational component and growth is temporarily unoriented. When growth of the cell in length is resumed, *dextral* spiral growth sets in at once. About an hour and a half later, dextral spiral growth regularly reverses and becomes *sinistral*. Sinistral orientation of growth is then normally maintained thereafter. This consistent sequence of changes in the orientation of growth emphasizes the existence of directional properties in the primary wall. The possibility that reversal of spiral growth is explicable in terms of a crossed spiral structure of the primary wall layers is discussed.

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NOTE ON PHOTOTROPISM IN PILOBOLUS¹

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DURING A series of lectures by Dr. A. H. R. Buller at Louisiana State University in the summer of 1941 a survey of the work on *Pilobolus*² was presented which directed attention to the highly specialized mechanism orienting the sporangia with respect to light, to the seeming importance of carotin in relation to such orientation and to the researches indicating that *Pilobolus* responded to the light of all the regions of the visible spectrum. Dr. Buller's presentation prompted the present inquiry.

That radiant energy has to be absorbed before it can become effective in stimulating a biological response is a precept of long standing known as the Grotthus law. Among green plants the phototropic response has been commonly observed to be incited by radiations of a wave-length shorter than about 5,400 Å. Absorption by carotenoids is typically negligible from 5,400 Å to 8,000 Å, so that from a physical standpoint an apparent association of these substances with phototropism is in line with the Grotthus precept. On the other hand, the reported bending of plants toward radiation of wave-lengths longer than about 5,400 Å is definitely disconcerting. With respect to *Pilobolus*, therefore, one might be tempted to suggest that either *Pilobolus* does not turn toward red light or that, if it does turn, there must be some pigment present which absorbs in the 5,400 Å to 8,000 Å range.

The light-sensitivity of *Pilobolus* has been studied by a number of investigators. The conclusions reached from the earlier researches were not in agreement and in an effort to clear up the muddled situation Parr (1918) carried out extensive studies at the University of Illinois, assisted by some members of the Physics Department. It appears to have been largely through these studies that the idea of a general sensitivity to radiations throughout the visible spectrum became favored for *Pilobolus*. The paper by Parr (1918) included a valuable survey of researches on *Pilobolus*, together with an excellent bibliography. More recently Bünning (1936-1937) carried out researches designed to appraise the valid-

ity of a proposed association between phototropism and carotenoids. In his first paper he questioned the method of Parr; yet upon adopting a preferred method he reported *Pilobolus kleinii* as sensitive to red light characterized by the wave-length range 6,500 Å to 6,900 Å. In a second paper he attempted to correlate the carotin content of *Phycomyces*, *Pilobolus*, and the *Avena* coleoptile with the degree of phototropic sensitivity of the respective involved structures. The substantial order of agreement appeared complicated by the revealed association of chlorophyll with carotin in the *Avena* coleoptile following exposure to light. In a third paper he reported failure to demonstrate with *Pilobolus* or *Phycomyces* a satisfactory agreement between the absorption curve of a specific carotenoid and the curve of phototropic response of the carotin-containing structure. With respect to green plants, his researches led him to implicate chlorophyll as associated with the phototropic response, an involvement which appeared to complicate the interpretation of phototropism in fungi.

The proposed sequence in the present series of experiments designed for a further study of the light-sensitivity of *Pilobolus* was as follows: (1) to verify the turning of *Pilobolus* to red light and (2) having verified the turning, to extract the pigment and determine the absorption characteristics within the range of visible radiation.

Before proceeding with tests involving *Pilobolus*, it seemed desirable to set up a beam of red light of measured wave-length range and intensity and to make certain that this beam incited no phototropic response in green plants. For this purpose a dark box was provided adjacent to the exit slit of a reflecting monochromator. A General Electric S-type single-coil filament lamp operated at 12 volts was the source of light. The slit widths were so adjusted as to yield a beam of radiation centered at 6,800 Å (wave-lengths ranging from 6,576 Å to 7,024 Å) with an intensity of the order of 800 ergs/cm². About 200 seeds of Italian rye grass were planted in a 400 ml. Pyrex beaker half full of moist soil. The beaker was so placed that germinating seedlings, upon reaching the top of the beaker, would extend

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² Part I of Volume VI of Dr. Buller's esteemed *Researches on Fungi* is concerned with the biology and taxonomy of *Pilobolus*.

into the horizontal beam of red light. Germination was practically 100 per cent. The growth of the seedlings in red light was uniformly upright and the plants were deep greenish orange-yellow, in contrast to the pale straw color of plants developed simultaneously in complete darkness. There was thus assured a horizontal beam of radiation that would promote chlorophyll development and yet be of such a quality as to fail to incite phototropic response.

Two types of experiments were then carried out with *Pilobolus*, one involving observations on bending or the orientation of the fruiting structures with respect to the direction of the horizontally-incident light, and the other involving observations on the disposition of discharged sporangial masses with respect to the radiation. In both types of experiments the initial procedures were the same. Dung balls obtained from a horse pasture were placed in moist chambers kept at moderate temperatures. As soon as *Pilobolus* became distinguishable, suitable individual balls were selected and removed to the experimental conditions.

In the experiments designed to provide for the observation of the bending or orientation of the fruiting structures the dung balls were suspended on fine wires over water in 400 m.l. Pyrex beakers, each wire being held to the underside of a superimposed Petri plate cover section by means of a bit of modeling clay. The covered beakers with enclosed balls were placed in the dark box in the horizontal beam of radiation.

When grown under the conditions above described and tested, the fruiting structures of the *Pilobolus*³ developed and became extended within the beam of red light, but in no instance was there any evidence of an orientation toward the horizontally-incident light source. The tests in the red light were observed after twenty-four hours, but were continued for forty-eight hours. They were repeated extensively, and in the aggregate they comprised hundreds of fruiting stalks. By way of contrast, similar tests were carried out in a beam of radiation centered at 4,600 Å, and marked orientation was observed in all fruiting structures in all tests at the end of twenty-four hours. Further tests indicated that, placed in this radiation at right angles to the beam, *Pilobolus* would become fully oriented toward the blue light source in about seventy minutes at 25°C.

In the experiments designed to provide for the observation of discharged sporangial masses, suitable dung balls were placed on moist filter paper in the center of ordinary Petri plates. An extra cover was inverted and placed beneath the plate so as to elevate the original cover and provide for suitable space to permit the discharge of the sporangial masses. The apparatus was placed in the horizontal beam, and the direction of the incoming light was indicated by an arrow marking. After seventy-two hours the plates were removed. The disposition of the sporangial masses was recorded by markings made on a

³ The material used in these studies appeared to be *Pilobolus longipes* van Tieghem.

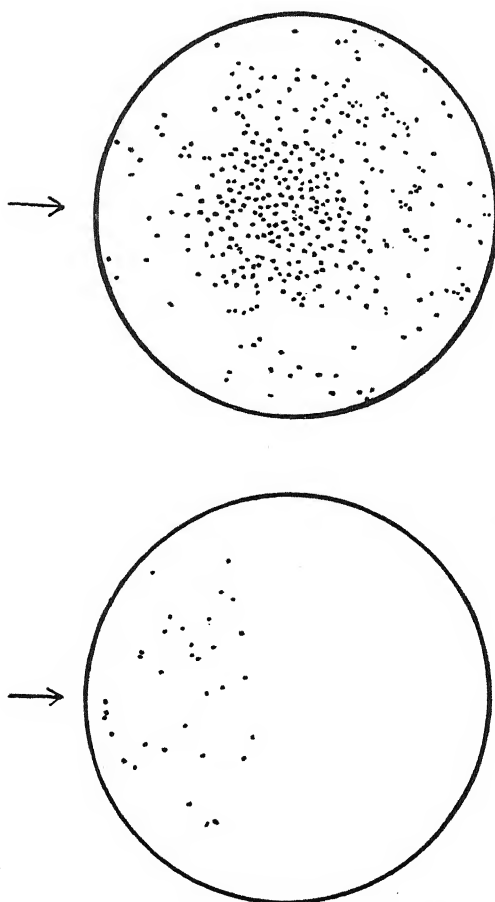


Fig. 1. Distribution patterns of *Pilobolus* sporangia. The fruiting structures were developed in light at horizontal incidence, the direction being indicated by arrows. Each dot marks the point of impact of a sporangial missile upon the superimposed Petri plate. Above, radiation centered at 6,800 Å; below, radiation centered at 4,600 Å.

piece of clear cellophane placed over the top cover of the Petri plate. The tracings indicated that there was no tendency of the discharged sporangial masses to present an enhanced frequency of distribution in the direction of incident radiation centered at 6,800 Å. The experiments of this type were repeated extensively and in the aggregate they also comprised hundreds of fruiting stalks. By way of contrast, similar tests were carried out in a beam of radiation centered at 4,600 Å. In these tests the distribution of the sporangial masses was such as to evidence a marked orientation of fruiting structures with respect to the direction of the incident radiation. The distribution patterns obtained with 6,800 Å radiation and with 4,600 Å radiation are shown in figure 1.

It was clear that these results removed the occasion for endeavoring to extract from *Pilobolus* a pigment absorbing red light. While the results were at variance with those reported by Parr (1918) and by Bünning (1936-1937), yet in indicating phototropic light-sensitivity as restricted to the short wave-

length range of visible radiation they indirectly appeared to give emphasis to the carotenoid-phototropism association so thoroughly studied by Bünning. Continuing, however, with *Pilobolus*, devoid of chlorophyll, demonstrable as having a phototropic light-sensitivity comparable with green plants, the results might also be interpreted as somewhat at variance with the chlorophyll-carotenoid alliance proposed by Bünning. In Zscheile's (1941) review of plastid pigments the absorption curves for various carotenoids were given. These curves indicated that absorption ceased within the range of 5,000 Å to 5,400 Å, which range also covered the variation found among the results of investigations involving the upper wave length limit of the phototropic response in green plants.

SUMMARY

When continuously exposed to radiation centered at 6,800 Å with a range 6,576 Å to 7,024 Å, an inten-

sity of the order of 300 ergs/sec/cm²., and demonstrable as non-phototropic to green plants yet promoting chlorophyll development, the fruiting structures of *Pilobolus* did not become oriented with respect to the source of light and discharged their sporangia at random. These results were not in agreement with those reported by Parr and by Bünning, yet they were consistent with the Grotthus precept and the association of carotenoids with phototropism.

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PRELIMINARY REPORT ON THE INTERGENERIC MATING OF THRAUSTOTHECA CLAVATA AND ACHLYA FLAGELLATA¹

S. B. Salvin

MANY SPECIES of the Saprolegniaceae combine asexual characters of two or more genera of that family. These include, for example:

(1) *Protoachlya* sp., in which the spores are diplanetic, "... on emerging ciliated, and all or some showing sluggish or less often active motion, some remaining attached in an irregular clump to the tip of the sporangium" (Coker, 1923);

(2) *Isoachlya* sp., in which the sporangia arise "either by cymose or pseudo-cymose arrangement, as in *Achlya*, or by internal proliferation as in *Saprolegnia*, both modes occurring earlier or later in the development of one and the same species, or frequently on the same main hypha" (Coker, 1923);

(3) *Achlya dubia* Coker, in which the spores emerge "as in *Achlya* or as in *Thraustotheca* in varying proportion, ... not rarely ... as in *Dictyuchus*" (Coker, 1923);

(4) *Thraustotheca primoachlya* Coker and Couch, in which the spores of the initially formed sporangia are "usually of the *Achlya* type, ... the spores discharging through an inconspicuous apical papilla or clustering at the tip," whereas the later sporangia burst "at the tip or sides, or in both places, the spores swelling out by degrees and the sporangial wall in large part disappearing" (Coker and Couch, 1924);

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(5) *Dictyuchus achlyoides* Coker, in which sporangia of "both *Achlya* and *Dictyuchus* types" are "abundant, the *Achlya* type appearing first, the *Dictyuchus* ones later" (Coker, 1927); and

(6) *Thraustotheca bispora* (Salvin, 1942) (= *Brevilegnia bispora* Couch; cf. Couch, 1927), in which the initial sporangia resemble "the *Achlya* type, many of which, however, lack a distinct papilla of dehiscence, a considerable part of the sporangial tip giving way for the exit of the spores," and in which the later sporangia are "of the same shape and size as the early ones but [dehisce] by the swelling of the spores and consequent bursting of the sporangial wall much as in the later sporangia of *Thraustotheca primoachlya*" (Couch, 1927).

The mixed characteristics of these species may be (1) an indication of their primitiveness, *i.e.*, they contain several characters which in later evolution may become separated in different forms, or (2) the result of intergeneric matings, *i.e.*, species with different sporangial characters may have fused sexually to give rise to generations which combine the qualities of the two parents. This latter possibility has never been demonstrated experimentally, *i.e.*, no one has ever successfully brought about the fertilization of the oospheres of one species with the antheridia of another. The writer, therefore, conducted experiments to determine whether such matings may be induced between two homothallic species with declinous antheridia. Although fifty-one combinations of species were tried, only one resulted in interspecific matings (fig. 1), namely, the combination of *Achlya flagellata* and *Thraustotheca clavata*. These two species were used in the subsequent

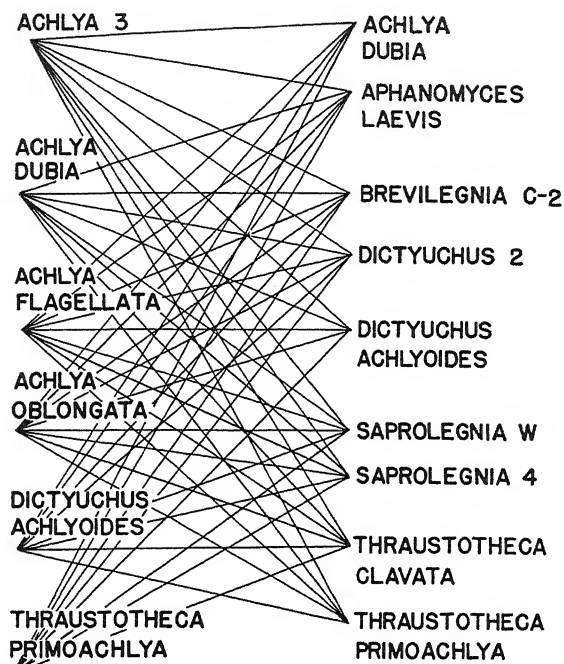


Fig. 1. Illustration of attempted interspecific matings.

experiments because they form many oogonia and diclinous antheridia in agar media when the culture is but a few days old, and their oospheres do not develop parthenogenetically. This combination of species was especially effective, since the early formation of oogonia in *A. flagellata*, occurring less than forty-eight hours after growth had begun, induced in *T. clavata* the development of antheridial branches before that of oogonia. Thus, it was possible to trace the oogonia and the attached antheridia to their respective sources, and accurately determine their specific origin. The main difficulty was in the fact that antheridial hyphae of *A. flagellata* were formed just prior to and simultaneously with the oogonia of that species and fertilized most of the enclosed oospheres.

MATERIALS AND METHODS.—Success in the mating experiments was first attained when the two fungi were inoculated on 25 cc. standard Difco corn meal agar about 6 cm. apart and diametrically opposite one another. When the respective mycelia grew toward one another and eventually became intermingled in a narrow band approximately midway between the two points of inoculation, examination of this band usually revealed several oogonia of *A. flagellata* partially enveloped by antheridia of *T. clavata*. Under these conditions, it was a long and difficult task to determine the origin of the sexual structures and subsequently to isolate them, especially since the antheridia of *A. flagellata*, which had appeared at the time of oogonium formation, fertilized most of the *Achlya* oogonia.

Therefore, attempts were made to find an experimental arrangement in which the origin of the antheridia and the oogonia from the two mated species

could be easily established and in which mature oospores, resulting from intergeneric matings, could be easily obtained in large numbers. Although the writer tried several methods of growing these two fungi in close proximity, none showed any improvement over the original one. However, some are worthy of further elaboration, because they were partly successful and might offer suggestions for further approach to the problem. The techniques (a) of growing the organisms on half-strength Difco corn meal agar as above and (b) of inoculating each fungus on a hemp seed and then placing the resulting young mycelia adjacent to one another in 15 cc. of water did not reduce the time nor the difficulty of experimentation. Since it was believed that if the mycelium were impeded by a wall perforated by a tiny hole, a few hyphae would proceed into the area beyond, a rectangular cup of thin cellophane, containing a tiny hole in the center of each of the longitudinal walls and half-filled with nutrient or non-nutrient agar, was placed midway between the points of inoculation of the fungi. Although a few hyphae of each of the inoculants entered the cellophane cup

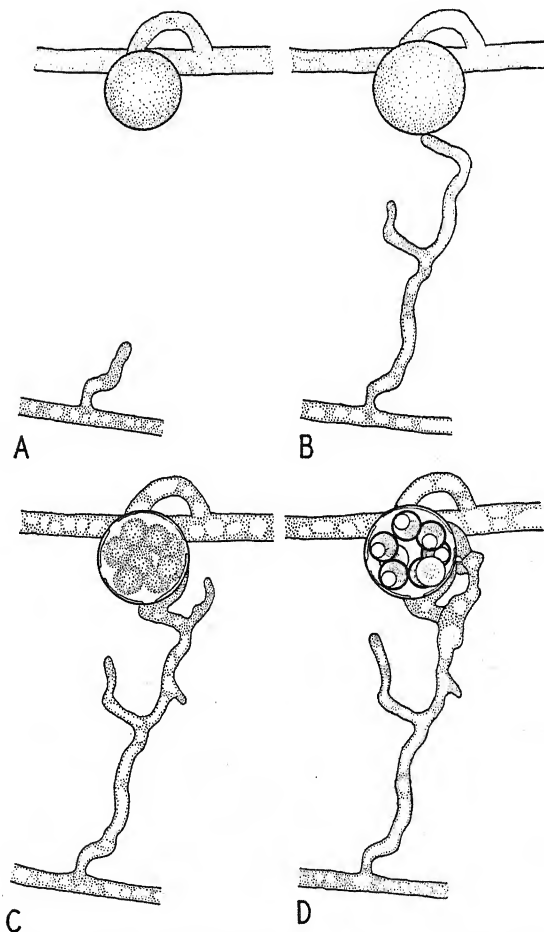


Fig. 2. Camera lucida drawings illustrating fertilization of an oogonium of *Achlya flagellata* by an antheridium of *Thraustotheca clavata*. $\times 300$.

through each of the perforations, these hyphae did not grow directly toward one another and bring about sexual fusion, but branched, curled, and spread about profusely, thus making the determination of the origin of the respective sexual structures even more difficult.

RESULTS AND DISCUSSION.—The writer was, therefore, driven by necessity to the long and arduous task of tracing the hyphae bearing the antheridia and oogonia to their source in order to determine accurately the point of origin of the respective sexual structures. Under these conditions, as the oogonia of *A. flagellata* developed alongside the hyphae of *T. clavata* in 25 cc. of Difco corn meal agar, antheridia of the latter species were formed and attracted to the adjacent oogonia (fig. 2, A, B). Eventually, these antheridia became attached, formed fertilization tubes, and apparently brought about fertilization, since oospores appeared shortly thereafter (fig. 2, C, D).

Although several cases of "imperfect hybridization" have been described in interspecific and intergeneric crosses in the Saprolegniaceae (Couch, 1926; Raper, 1939) and the Mucoraceae (Blakeslee, 1915), the successful mating of two separate homothallic species of the Saprolegniaceae has never been reported. The foregoing results, therefore, are the first to indicate that sexual fusion may occur between the homothallic thalli not only of two separate species but also of two separate genera.

However, until the oospores are germinated and the characteristics of the resulting generations studied, the experiments do not prove conclusively that sexual or nuclear fusion took place between the two species. When attempts were made to germinate under ordinary environmental conditions the oospores resulting from the intergeneric matings, not a single oospore, when transferred to nutrient agar, gave rise to a vegetative mycelium. Therefore, in order to stimulate this germination, oogonia containing these oospores were removed from the agar about a week after their maturation and subjected to (a) drying at room temperature for two weeks, (b) drying for

a week and heating to 100° F. for six to seven hours, and (c) drying for two to three weeks at a temperature of 5° C. Subsequently, when the treated oogonia were placed in a nutrient agar, no germination followed. This failure to germinate may be attributed to the lack of proper conditions, although there is the alternate possibility that these oospores are non-viable.

In spite of the failure to induce germination of the oospores, the significance of these intergeneric matings of two homothallic species is great. In the first place, these matings indicate that sexual fusion in nature may well occur between homothallic species of the same or different genera of the Saprolegniales. In the second place, this intergeneric fusion suggests that species with intermediate asexual characters may have originated from interspecific hybridization. Also, the sudden appearance of an atypical method of asexual reproduction in a given species may be similarly explained. In the third place, there is the indication that the modern concept of a genus in the Saprolegniaceae is possibly inadequate in that it does not take into consideration the possible mingling of characters as a result of intergeneric matings. Finally, there may well be a closer phylogenetic relationship between some of the genera of that family than is at present recognized.

SUMMARY

That many species of the Saprolegniaceae have asexual characters which are typical of or intermediate between two or more genera of that family may be the result of intergeneric matings. When attempts were made to mate species of different genera, one proved successful, namely, that between *T. clavata* and *A. flagellata*. However, the oospores resulting from this intergeneric fusion did not germinate, probably because of the lack of proper conditions therefor.

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WATER UPTAKE BY EXCISED ROOT SYSTEMS OF THE TOMATO DUE TO NON-OSMOTIC FORCES¹

J. van Overbeek

THE CLASSICAL concept of water uptake into cells by means of osmosis is so universally accepted that it is often regarded more as a fact than a convenient theory. Root pressure also is often considered to be entirely osmotic in nature (Sabinin, 1925; Crafts and Broyer, 1938). However, experiments are known which make it appear as if osmosis is inadequate to explain the water uptake of cells. Ursprung and Blum's experiments (1921, 1925) seem to show that water uptake by the cells of the endodermis is possible against an osmotic gradient. Bennett-Clark and Bexon (1940) have experiments which tend to show that the "water absorbing power" of the leaf cells exceeds the osmotic pressure of the vacuole. Heyl (1933) concluded from a study in which he investigated the effect of temperature upon the rate of bleeding and the rate of backward movement of the exudate under influence of plasmolytica, that the mechanism of bleeding cannot be exclusively osmotic in nature.

The purpose of this investigation was to find out experimentally whether or not osmosis alone is capable of explaining root pressure. In order to do this, a simple basic experiment was designed by which it was possible to measure (A) the osmotic pressure of the exudate² and (B) the pressure with which water is taken up by the roots. If these two values are equal in magnitude, root pressure can be explained on the basis of osmotic pressure alone. If the pressure with which water is taken up by the roots (root pressure) is larger than the osmotic pressure of the exudate, then root pressure is due to other causes in addition to osmosis.³

The root pressure was determined as the osmotic pressure of a mannitol solution which exactly compensated the root pressure.

TECHNIQUE.—Seeds of "San Jose Canner" tomatoes were sown in sand in flats and watered with Hoagland solution. After the seedlings were six to eight weeks old, they were transferred to culture solutions with forced aeration in one of the air-conditioned greenhouses (26.5°C. and 70 per cent humidity). For constancy of temperature and other properties of these greenhouses, see Went (1942). In order to avoid "shock" reactions the experiments (with the exception of the one mentioned in fig. 3) were carried out at the same *constant conditions* as those under which the plants were grown. The in-

dividual plants were held with non-absorbent cotton in paraffined wooden lids, which were placed on one-gallon mayonnaise jars which were painted white over a black undercoat and filled with Hoagland solution (Hoagland and Arnon, 1938). In order to obtain plants rich in carbohydrates which would be capable of carrying on metabolism for a considerable time after they were decapitated, the nutrient solution was not renewed, but the water lost by transpiration was replaced by distilled water. In this way low-salt plants which are rich in carbohydrates (Hoagland and Broyer, 1936; Grossenbacher, 1938) were obtained. The pH was adjusted periodically and maintained between 5 and 6, and ferric chloride was added weekly. The Hoagland solution was renewed in a few special experiments which will be mentioned later. In the experiments mentioned in the last section, dealing with the effects of KCN, a nutrient solution suggested by Arnon and Hoagland (1940, p. 477) was employed. Exceptional root growth was obtained in this solution and the pH adjustments were not necessary. Iron citrate was given twice weekly.

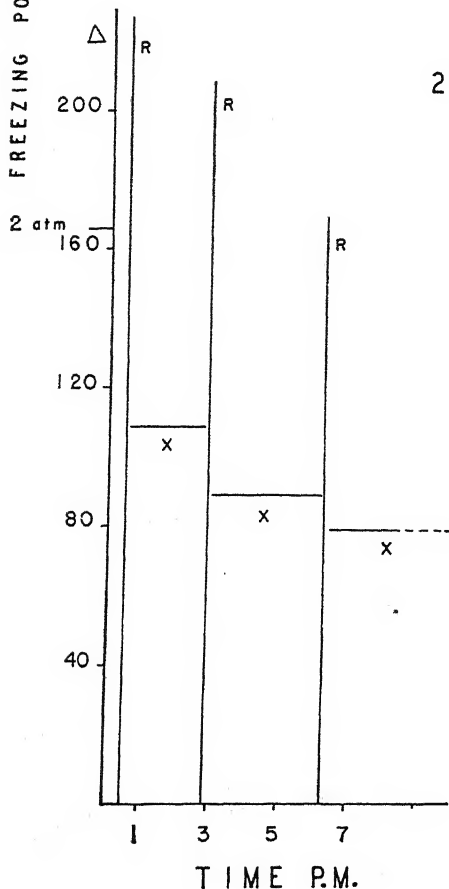
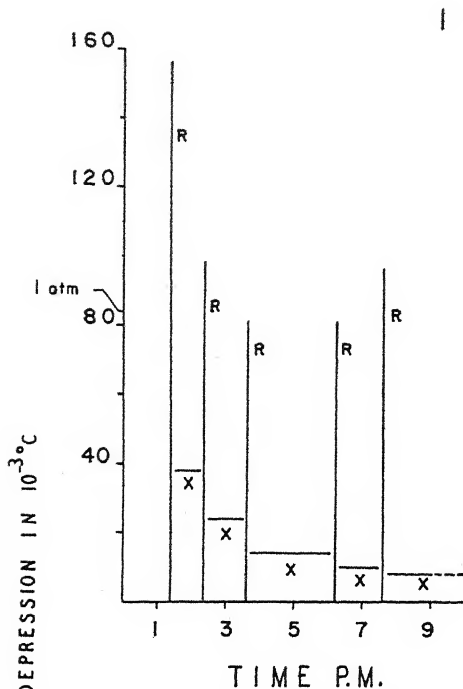
After the plants had grown from four to seven weeks in the culture solution, they were cut about one to two inches above the uppermost roots. This decapitation was done about noon, because it was found that plants cut earlier in the day often did not bleed at all, even when left for a day or longer. After decapitation the plants were transferred to distilled water. In some experiments distilled water was put in the culture jars a few days before the plants were decapitated (fig. 1). The root system was put in *distilled water* because it eliminates accumulation of solutes as a complicating factor in the process of exudation; it also increases the rate of exudation over that of root systems kept in nutrient solution. In some experiments (fig. 2) the roots were kept in a full-strength Hoagland solution instead of in water. This did not influence the results, however. It only caused a slower rate of exudation and a higher osmotic pressure of the exudate. During the experiments, as well as during the growing period, forced aeration was used continuously.

The stump of the plant was attached to a thick-walled U-tube with narrow bore, by means of a small piece of rubber tubing firmly attached to the stump with string. In this manner a perfectly water-tight connection was obtained which allowed the U-tube to be interchanged conveniently for a horizontal "potometer" tube (for rate determinations). The majority of plants bled profusely, often at a rate of 2 cc. per hour. After a minimum of 2 cc. of the exudate was collected for freezing point determinations, the U-tube was replaced by a horizontal thick-walled tube (inside diameter 0.042 inches). The meniscus moved rapidly outward.

¹ Received for publication April 18, 1942.

² The nomenclature regarding osmotic quantities proposed by Meyer (1938) and Meyer and Anderson (1939) is used throughout. Therefore "osmotic pressure" of the exudate denotes "osmotic value" or "osmotic concentration" of the older literature.

³ It is assumed that the osmotic pressure of the exudate equals, or at least is not higher than, that of the contents of the vessels down in the water-absorbing region of the roots. Evidence for the correctness of this assumption is given later in the paper.



Next a concentrated mannitol solution was carefully added to the distilled water in which the roots were immersed. Mannitol is an osmotically active but otherwise a physiologically inert substance which penetrates into cells with great difficulty (Colander and Bärlund, 1933), and for this reason is to be preferred to substances such as NaCl. At a concentration just sufficient to stop the bleeding, the osmotic pressure of the mannitol solution equals the pressure with which the roots take up water (root pressure). A rapid circulation of the solution was obtained by means of forced aeration. Mannitol was added until the meniscus remained stable for five minutes. If too much mannitol was added, the meniscus moved inward, and some of the solution in the 600 cc. beaker in which the roots were immersed was poured off and replaced by water until the "compensation point" was reached. "Rebound" effects such as reported by Rosene (1941a) in onion roots were also observed in the tomato roots. These effects may be due to cortical and other cells between the xylem and outside solution coming into equilibrium with each other and their surroundings. In order to avoid errors due to such "rebound" effects a total of fifteen minutes was allowed for finding the mannitol concentration which just stopped bleeding. The method is very sensitive, and the effect of a single drop of concentrated mannitol solution added to the solution in the 600 cc. beaker may be noticed in the movement of the meniscus in the horizontal tube. The method has an advantage over methods determining the rate of bleeding in that changes in the water permeability of the root cells do not affect the root pressure determinations.

After the mannitol concentration was found which would stop water uptake by the roots, a sample of the mannitol solution was taken for *cryoscopic analysis*, the roots were rinsed and placed in fresh distilled water and some more exudate was collected. The entire procedure was repeated several times. In later experiments (fig. 1) root pressure measurements were made immediately after the plants were decapitated. In earlier experiments (fig. 3) a sample of exudate was collected before the first root pressure measurement was made. It was often possible to make five root pressure determinations and to collect four samples of exudate within seven hours after the plant was decapitated (fig. 1). The osmotic pressure of the exudate was measured cryoscopically with a small Beckman apparatus with which samples as small as 2 cc. could be analyzed. In doing the

Fig. 1-2. Root pressure compared with osmotic pressure of the exudate.—Fig. 1. The pressure with which an excised root system takes up water (vertical lines R) exceeds the osmotic pressure of the exudate (horizontal lines X) considerably. Pressures expressed in the corresponding freezing point depressions as well as in atmospheres. During the time the exudate was collected and twenty-eight hours prior to the beginning of the experiment the roots of the plant were kept in aerated distilled water.—Fig. 2. Experiment similar to that of figure 1, except that during the experiment and prior to it the plant was kept in full-strength Hoagland solution.

freezing point determinations great care was taken to under-cool always to the same point ($-2^{\circ}\text{C}.$). In this way highly comparable results were obtained. The standard error of the Δ (freezing point depression) determinations was $0.004^{\circ}\text{C}.$ Differences in Δ larger than $0.010^{\circ}\text{C}.$, therefore, can be considered significant. All the Δ determinations of a single experiment were carried out at its conclusion. Samples were saved in the refrigerator until they were analyzed.

RESULTS WITH LOW SALT PLANTS.—In practically all experiments the root pressure, as measured by the compensation method with mannitol, exceeded the osmotic pressure of the exudate. In figure 1, as in the other figures, the root pressure is indicated by vertical lines (marked R) and the osmotic pressure of the exudate is indicated by horizontal lines (marked X). The length of the horizontal lines indicates the time during which the sample was collected. The plant for which the data are shown in figure 1 was decapitated at about 1 P.M., and immediately after the necessary apparatus was attached, the pressure with which water was taken up was measured (first vertical line R). During the next forty minutes 2 cc. of exudate were collected, the freezing point depression of which was $0.088^{\circ}\text{C}.$ as against $0.156^{\circ}\text{C}.$ for the mannitol solution which just checked the bleeding (R). If expressed in atmospheres, it means that the root pressure was 1.88 atm. and the exudate had an osmotic pressure of 0.46 atm. A second root pressure determination immediately following the collection of the first exudate proved to have a Δ of $0.098^{\circ}\text{C}.$ (1.18 atm.). During the next hour, following the second root pressure determination, another 3 cc. of exudate was collected having a Δ of $0.24^{\circ}\text{C}.$ (0.29 atm.). During the next hours the freezing point depression of the exudate decreased still more, reaching $0.008^{\circ}\text{C}.$ during a period which lasted from 7:35 P.M. to 6:45 A.M. the next morning. During this final period 14 cc. of exudate were collected. Although the Δ of the exudate dropped to $0.008^{\circ}\text{C}.$, the pressure with which water was taken up by the roots never dropped below a value ten times as high ($0.080^{\circ}\text{C}.$). From May 25 to June 11, nineteen root pressure determinations and seventeen exudate determinations were made within a period of seven hours after the plants were decapitated and under conditions (roots in distilled water) similar to those shown in figure 1. The average Δ of the exudate was $0.033 \pm 0.004^{\circ}\text{C}.$ (0.40 atm.). The average pressure with which the roots took up water was $0.116 \pm 0.007^{\circ}\text{C}.$ (1.40 atm.). The average difference between these two values was $0.083 \pm 0.005^{\circ}\text{C}.$ (1.00 atm.). This is the pressure with which the roots take up water in excess of osmotic pressure,³ and it is on the average 2.5 times as large as the osmotic pressure under the conditions of this type of experiment.

RESULTS WITH HIGH SALT PLANTS.—Since the results discussed above were obtained with roots which were kept in distilled water, the question arose whether similar results could be obtained if the roots

were kept in full-strength Hoagland solution. Figure 2 clearly shows that this was the case. Three days prior to decapitation the plants were given full instead of half-strength Hoagland solution. On June 2 at 12:15 P.M. a plant was decapitated and the root pressure was determined (fig. 2). It proved to be 2.75 atm. ($\Delta=0.227^{\circ}\text{C}.$). In a parallel experiment the root pressure determination made immediately after the decapitation was 3.50 atm. This was the highest value for root pressure ever found in these experiments. Not only the root pressure but also the osmotic pressure of the exudate was much higher in the high salt than in the low salt plants. In the experiment shown in figure 2 the osmotic pressure of the exudate in consecutive periods was respectively 1.32 atm. (corresponding to a Δ of $0.109^{\circ}\text{C}.$), 1.07 atm. ($\Delta=0.089^{\circ}\text{C}.$) and 0.95 atm. ($\Delta=0.079^{\circ}\text{C}.$). The amount of exudate collected during these periods was respectively 4, 3 and 7 cc., the last period lasting twenty-two hours. In a parallel experiment almost identical figures were obtained. Thus, the exudate is more concentrated than the full-strength Hoagland solution itself which had a Δ of approximately $0.070^{\circ}\text{C}.$ A further comparison between figures 1 and 2 shows that the "active" pressure,⁴ which is the difference between the root pressure and the osmotic pressure of the exudate is about equal in both high and low salt plants. The root pressures found in these experiments compare well with the ones found by Kramer (1941) who concluded that the root pressure of tomatoes that stopped bleeding due to drying out of the soil must be about 1 or 2 atm. White (1938), however, reported root pressures as high as 6 atm. in isolated tomato roots cultivated *in vitro*.

RESULTS OF EXPERIMENTS DONE OVER A PROLONGED TIME.—The experiments discussed above were discontinued after about one day, and the bulk of determinations were made within about seven hours. Tomato plants, however, may continue to bleed for longer periods (Grossenbacher, 1938, 1939; Skoog, Broyer and Grossenbacher, 1938; Heyl, 1933). Figure 3 may serve as an example. The plant was grown at $20^{\circ}\text{C}.$ and removed just prior to decapitation to $26.5^{\circ}\text{C}.$ where it stayed for the duration of the experiment. On April 22 at 12 noon the plant was decapitated and 15 cc. of exudate were collected during that day, 20 cc. were collected during the next period at the end of which the root pressure was determined (vertical line R). This procedure was repeated until April 25 noon. It is obvious from figure 3 that the osmotic pressure of the exudate has remained practically constant during the three days of the experiment. The root pressure, however, went through cycles of high and low pressures, the high pressures coming about noon. In at least three other experiments similar relatively high root pressures were found at noon, which is in agreement with Grossenbacher (1938), who reported high rates of

⁴ The part of the root pressure that cannot be accounted for on the basis of osmosis has been arbitrarily called "active" pressure to set it apart from the simple physico-chemical phenomenon of osmosis. The "active" pressure can be inhibited by KCN and the osmotic pressure cannot.

exudation occurring during that time. Although the diurnal cycle was not made a special object in this study, it seems reasonable to conclude that the cyclic increase and decrease in rate of exudation reported by Grossenbacher (1938, 1939), Heyl (1933) and many others is due to a periodic increase of the "active" part of the root pressure and not to the osmotic part. However, this may not explain the periodic increases in rate quantitatively, since it is also possible that periodic changes in water permeability exist.

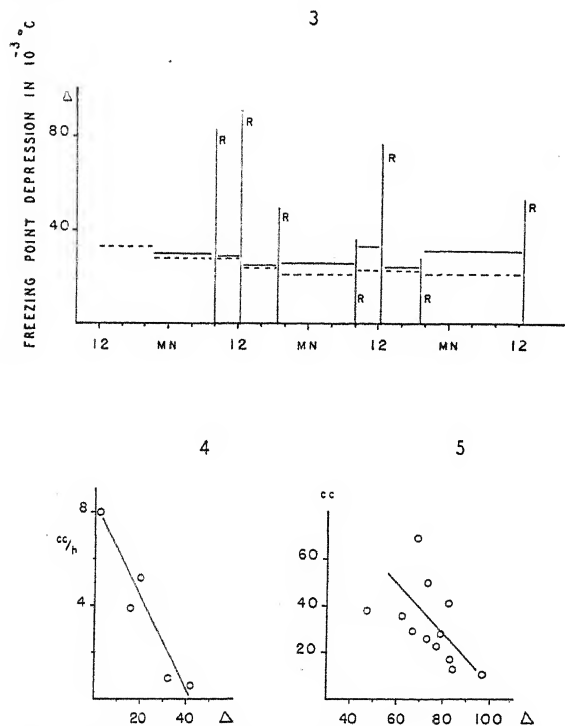


Fig. 3-5.—Fig. 3. Experiment similar to that of figure 1, but root pressure and exudate determinations were continued for three days. The osmotic pressure of the exudate was determined cryoscopically (solid horizontal lines) as well as by calculation from the conductivity (broken lines).—Fig. 4. Negative correlation between the rate of bleeding (ordinate) and the osmotic pressure of the exudate. The latter expressed in the corresponding 10⁻³°C. freezing point depression (abscissa). The rate of bleeding was controlled by suction on the cut stem. Root system in distilled water during the experiment.—Fig. 5. Negative correlation between the total amount of exudate collected per plant in one week (ordinate) and its average freezing point depression (solute concentration; abscissa). Tomato plants grown in subirrigated gravel culture.

COMPOSITION OF THE EXUDATE.⁵—Except in the first sample of exudate, collected shortly after decapitation, sugars were absent in the exudate. In the first sample both glucose and sucrose were found in concentrations which rarely exceeded 0.01 per cent. This agrees with the findings of Crafts (1936)

⁵ I wish to express my thanks to Mr. E. L. Grady who carried out the sugar analyses and to Mr. Alan Bell who did the conductivity determinations.

that no phloem exudate appeared more than a few hours after a cut had been made. Conductivity measurements were carried out on a large number of samples taken during the earlier stages of the experiments. On the assumption that the osmotically active material of the exudate has a conductivity equivalent to KCl, the Δ was calculated from conductivity data. For forty-three instances the calculated Δ was on the average only 0.005 ± 0.001 °C. less than the actual Δ . This calculated Δ is drawn into figure 3 as dotted horizontal lines as compared with the solid lines, which represent the actually determined freezing point depressions of the exudate. The exudate, therefore, may be considered to consist almost exclusively of electrolytes.

The pH of the first sample of exudate was between 5.5 and 6.0 and increased with the time after decapitation. Two days after decapitation the pH often was slightly greater than 7.0. Plants that still bled after eight days were found to have a pH of 8.5. The exudate of plants grown in gravel and subirrigated with Hoagland solutions which were adjusted daily to pH 3, 5, 4.5 and 5.5 had the same pH of 5.5 regardless of the pH of the medium. The gradual increase in pH with time after decapitation was the same as in plants grown in culture solutions. However, the concentration of the exudate, as determined by the freezing point depression, is dependent upon the rate of bleeding. A slowly bleeding plant usually has a more concentrated exudate than a fast bleeding one (fig. 4 and 5).

RELATION BETWEEN THE RATE OF BLEEDING AND THE Δ OF THE EXUDATE.—The conclusion that the difference between the root pressure and the osmotic pressure of the exudate represents the pressure with which water is taken up by "active" forces stands or falls with the assumption that the exudate represents the sap in the vessels of the water-absorbing region of the root. One could assume that the "active" pressure is only apparent because the Δ of the exudate might be far lower than that of the sap in the vessels of the root. One could suppose that this condition could be brought about by absorption of solutes in the course of the upward movement of the xylem contents. This is unlikely, however, because accumulation of solutes by the roots was excluded by placing the roots in distilled water during the experiments. Furthermore, if during the experiments solutes were removed from the xylem, one would expect that a slowly bleeding plant would have a less concentrated exudate than a faster bleeding plant. Exactly the reverse is true. This is shown in figure 4 where the rate of bleeding was varied by using suction (Kramer, 1933). A slow-moving exudate was actually more concentrated than a fast-moving one, which may indicate that some solutes were given off into the xylem. In agreement with this is the observation that at the end of prolonged experiments or after severe treatment with KCN the root pressure always was somewhat below the osmotic pressure of the exudate (fig. 6B). As long as the root system was still alive the root pressure remained at this

level and never was found to drop to zero, not even under conditions of severe irreversible narcosis. This is in agreement with Rosene (1941b) who observed the same for the rate of bleeding. The semi-permeability of the membranes was still intact under these conditions because the rate of bleeding could be changed by changing the concentration of mannitol solutions around the roots. The above is interpreted to mean that in old plants and in narcotized plants the root pressure equals the osmotic pressure of the sap of the xylem of the roots, that the osmotic component of the root pressure remains and that the "active" pressure has disappeared.

FURTHER EXPERIMENTS SHOWING THE DUAL CHARACTER OF ROOT PRESSURE.—The conclusion that the root pressure is made up of two components, (1) osmotic pressure and (2) "active" pressure, would be considerably strengthened if the components could be reversibly separated. The "active" pressure might be assumed to depend upon respiratory processes just as the accumulation of solutes by the roots (Hoagland and Broyer, 1936) or by other tissues (Steward, Stout and Preston, 1940). If the assumption is correct, one might be able reversibly to cut out the "active" component of the root pressure by using KCN. Figure 6 shows that this can be done. The plants used for these experiments were grown for $2\frac{1}{2}$ weeks in the solution suggested by Arnon and Hoagland (1940, p. 477) and had a very vigorous root system. Fifteen minutes after a plant was decapitated and its root system transferred to distilled water the root pressure was measured by means of the mannitol method described above. Immediately after this determination the roots were transferred to 10^{-4} M KCN (fig. 6A). The solution was stirred by bubbling air through it. After one hour the rate of bleeding had dropped to 50 per cent of its original value and mannitol was added to the KCN solution in order to determine the root pressure of the plant in this condition. It had dropped considerably as can be seen in figure 6A. Immediately after this root pressure determination the root system was removed from the KCN solution, rinsed and replaced in distilled water. One hour later the root pressure had returned to almost its original value. After this final root pressure determination, 4 cc. of exudate was collected in three hours, which had a Δ of 0.015°C .

A parallel experiment gave identical results. The first root pressure determination gave a Δ of 0.145°C . The root pressure after one hour in 10^{-4} M KCN gave a Δ of 0.077°C ; one hour after the root system had been returned to distilled water it was 0.125°C . In the following three hours 6 cc. of exudate were collected having a Δ of 0.020°C . The rate of bleeding of this plant was originally 150 arbitrary divisions of the potometer tube. After one hour in 10^{-4} M KCN it dropped to 70, to recover to 170, one hour after the roots were brought back in distilled water.

Figure 6B shows a similar experiment except that a ten times higher KCN solution was used for a

shorter period of time. The root pressure was reduced to less than 25 per cent of its original value, which is slightly below the osmotic pressure of the exudate. From experience with relatively high concentrations of HCN one could say that, even if the roots were kept for a longer time in this KCN solution, the root-pressure would have dropped no more, but this drop would not have been reversible as it was in this experiment (fig. 6B).

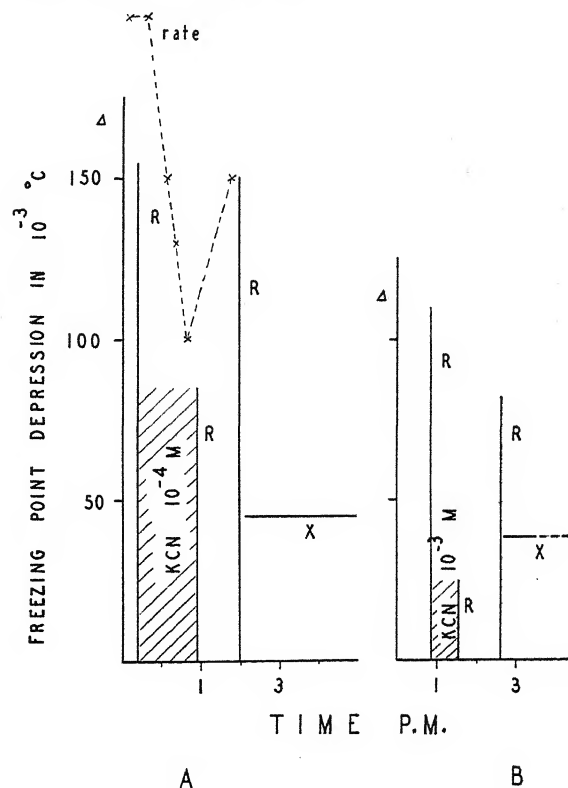


Fig. 6 A-B.—Fig. 6A. Reversible reduction of part of the root pressure by means of immersion of the root system in 10^{-4} M KCN for one hour (shaded part of the diagram). The rate of bleeding during the experiment is indicated by a broken line. Plants decapitated thirteen minutes before the first root pressure determination was started.—Fig. 6B. Same as figure 6A except for concentration and time of exposure to KCN. Even during severe exposure to HCN when the reduction in root pressure is no longer reversible, the root pressure was never found to drop completely to zero, as long as semi-permeability is retained.

DISCUSSION.—The great difference between the osmotic pressure of the exudate and the pressure with which the excised root system takes up water (fig. 1, 2, 3) makes it impossible to explain the entire phenomenon of root pressure on the basis of osmosis. The reversible separation of the "active" component of the root pressure by KCN makes it seem very likely that part of the water uptake of roots is connected with respiratory processes or at least with reactions involving heavy metal catalysis. In this connection it may be instructive to draw a parallel between this form of water uptake and the accumula-

tion of solutes by roots. Hoagland (1936) and Hoagland and Broyer (1936) showed that accumulation of solutes by excised roots is greatly dependent upon aerobic respiration. Similarly Steward (1933) showed that only strongly metabolizing cells of potato disks are capable of accumulating KBr. Rosenfels (1935) showed a close connection between respiration and solute accumulation in *Elodea*. What the actual mechanism of the "active" water uptake by roots really is remains a mystery for the time being. However, Keller (1930) in the second part of a voluminous paper dealing with water transport as an electrical phenomenon, believes that root pressure is due to electro-osmosis. The prerequisite for electro-osmosis is a potential difference across a membrane, a condition which may very well be realized in actively metabolizing root cells. Blinks (1940) has shown that the potential difference across the protoplasm of cells of *Halicystis* is distinctly under metabolic control (low oxygen, high CO₂, light, poisons, etc.). Blinks (personal letter), however, considers electro-osmosis unlikely because the current flow is probably not sufficient and the salt concentrations in cells are unfavorably high for electro-kinetic phenomena.

SUMMARY

The osmotic pressure of the exudate of decapitated tomato plants, grown in culture solution, is considerably lower than the pressure with which the roots absorb water. The latter pressure (root pressure) was determined as the osmotic pressure of a mannitol solution, placed around the roots, which reduces the rate of exudation exactly to zero.

It is concluded that the pressure with which healthy root systems of decapitated tomato plants absorb water is made up of two components: (1) an "active" pressure,⁴ and (2) a pressure of osmotic origin. Roots kept in distilled water during the experiment developed an average root pressure (determined cryoscopically by means of mannitol solutions) of 1.40 atm., of which 71 per cent was "active" pressure and 29 per cent pressure of osmotic origin. Roots kept in Hoagland solution during the experiment absorbed water with an average pressure of 2.68 atm., 48 per cent of which was "active." The active component of the root pressure could be reversibly eliminated by means of KCN.

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RESISTANCE OF WATERMELON TO THE WILT DISEASE¹

Albert E. Braun

THE SEEDLINGS of most edible varieties of watermelon, *Citrullus vulgaris* (Schr.), are highly susceptible to the wilt disease caused by *Fusarium oxysporum* f. *niveum* (E. F. S.) Snyder and Hansen, except those which have been bred for resistance by crossing with the Citron, a non-edible, less susceptible variety.

In a previous investigation to ascertain the causes of susceptibility to the wilt disease, Braun (1937) found no evidence that differences in susceptibility were due to anatomical variation. With this in view it was thought that differences in susceptibility might be due to the presence or relative abundance of a chemical compound.

Two varieties of watermelon were chosen for this study: A very susceptible variety, Kleckley Sweet (not the improved Kleckley Sweet) and a less susceptible variety, Citron. The Citron is not immune to the wilt disease, but the rate of growth of the fungus is very slow in the Citron as compared to the more susceptible varieties. Plants were grown in the field and harvested when six weeks old, dried in an oven at 60°C. and ground to a fine pulp. Stems and roots were kept separate. Samples of the pulp were refluxed in water for three hours and small portions of the water extract were placed in liquid culture media inoculated with *Fusarium oxysporum* f. *niveum* spores. After two weeks the cultures were examined, and the medium with the Citron shoot extract contained considerably less growth than the control. No differences from the control were observed in the cultures with the Citron root extract, the Kleckley Sweet root extract nor the Kleckley Sweet shoot extract. The sample pulp samples were dried and refluxed with ether for three hours. The ether was decanted and allowed to evaporate. The fatty material was suspended in water, and samples of each extract were placed in culture media and inoculated with *Fusarium oxysporum* f. *niveum* spores. None of the material extracted with ether had any influence on the growth of *Fusarium oxysporum* f. *niveum*. These results indicated that the water soluble extract contained a substance which has some relationship to susceptibility, and further tests showed that the material was a volatile acid. To determine the nature of the volatile acid, 500 g. of pulp of each variety were placed in separate distilling flasks, and water slightly acidified with sulfuric acid was added. One liter was distilled from each flask, neutralized with sodium hydroxide and

evaporated to the dry salt. From this was obtained 0.126 g. of salt from the Citron shoot distillate and 0.017 g. of salt from the Kleckley Sweet shoot distillate. The salts were analyzed for their acid constituents according to the method of Schickstanz, Steele, and Blaisdell (1940). The salt obtained from the Kleckley Sweet variety when distilled with toluenesulfonic acid in benzene or toluene gave no significant titration with 0.1 N sodium hydroxide. However, the salt obtained from the Citron variety when distilled with toluenesulfonic acid and benzene gave results as shown on table 1. The amount of acid per fraction indicates the presence of acetic acid. However, under the conditions of isolation it cannot be claimed that acetic acid is found in the free state in the plant. No significant titration was obtained with toluene.

TABLE 1. Fractional azeotropic distillation of the mixture in benzene; titrated with 0.1N sodium hydroxide. (Acid liberated from sodium salt with toluenesulfonic acid.)

Volume of fraction	0.1N NaOH
ml.	ml.
10	2.55
10	1.00
10	0.75
100	6.50
30	1.80
90	1.35

The fractional distillates which had been neutralized with sodium hydroxide were evaporated to dryness and redissolved in water slightly acidified with sulfuric acid and distilled. A sample distillate from each solution was tested for its action on the growth of *Fusarium oxysporum* f. *niveum*, but only the solution from the Citron shoot retarded the growth of the fungus. It is well known that the pathogen enters the root system of the seedlings, becomes established there and initiates the disease in both the susceptible variety and the more resistant Citron. The growth of the fungus in the Citron is apparently retarded by something from the shoot, which may be acetic acid, and not from the root itself, for the pathogen becomes established in the root system of either variety.

The action of acetic acid was also tested on the growth of the fungus, and it was found that one part of acetic acid in one hundred parts of nutrient solution completely inhibited the growth of the fungus,

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The author wishes to thank Mr. Donald Bolin of the Agricultural Chemistry Department, University of Idaho, for suggestions and the use of certain equipment.

while one part of acetic acid in five hundred parts of nutrient solution slightly retarded the growth of the fungus.

SUMMARY

In this investigation more acetic acid was found in the Citron, a variety of watermelon less susceptible to the wilt disease, than in Kleckley Sweet, a more susceptible variety. However, further investigations will be necessary to prove definitely that the acetic acid is the basis of resistance to the wilt disease.

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POSTGLACIAL MIGRATION OF FIVE FOREST GENERA ¹

Paul B. Sears

THE FOLLOWING study presents evidence concerning the postglacial movements of *Quercus*, *Tsuga*, *Carya*, *Fagus*, and *Tilia*. The area under consideration extends eastward from Minnesota and Iowa to the Atlantic Coast, and from the St. Lawrence Valley southward to Virginia and Tennessee.

The evidence has been secured by determining the relative order of appearance of the pollen of the above genera in 117 bog profiles, taken from 111 bogs distributed over the area. Of these profiles, eighty-five are from the published work of others, while thirty-two are based upon analyses made by the author or under his direction. Two of the thirty-two analyses have been published, thirteen are in press, and the remaining seventeen are on file at Oberlin.

The data for each of the five genera are mapped separately, the numbers 1 to 5 being used as symbols. The position of the symbol on the map indicates the location of the profile in question. The number itself indicates the order in the sequence, beginning at the bottom of the profile, of the first trace of pollen of the genus. Where two or more kinds of pollen make their first appearance at the same level, they are given the same number.

Within the limitations of the procedure, each map should indicate: (a) the areas of first appearance of the genus after the beginning of sedimentation and (b) the direction and extent within the area of subsequent movement. It must be emphasized that this procedure is tentative, as any reconstruction of the past must be. It should, however, open up a useful approach and point the way for critical and intensive local studies.

In addition, this information regarding sequence and direction of movements is here correlated with the results of a paper now in press (Sears, 1942), dealing with postglacial forest sequences within the area. Both papers continue investigations begun in 1916 on the historical factor in natural vegetation (Sears, 1926). The paper now in press reports fairly consistent evidence from Illinois, Indiana, Michigan,

and Ohio of the existence in postglacial times of two intervals of diminished mesophytism, separated by a relatively more mesophytic interval (see also Auer, 1930; Sears, 1931).

Since such apparent lessening of mesophytism seems to represent reversal or interruption of the normal course of forest succession, I have designated it as "retrogression" (Cowles, 1901). And since it is widespread, I have inferred that it was due, in both instances, to a decrease in available moisture. The resulting scheme is as follows:

- V. The present—probably cooler and with more available moisture than in IV.
- IV. A warm dry period—maximum of oaks and hickories, minimum of beech.
- III. A more humid, also warm, period—maximum of beech, and, in places, of hemlock.
- II. A dry, probably warmer, period—maximum of pine, often with oak.
- I. A moist cool period—maximum of fir and spruce.

The Roman numerals are here used as in the preceding paper, and are to be so understood hereinafter.

Allowance must be made for numerous and considerable sources of error, of which the following appear to be the most serious:

(1) Most analyses are based upon a single boring, although often checked with (unpublished) parallel borings. However, where two analyses of the same deposit do not agree in the exact order of appearance of the five genera, both have been mapped.

(2) The analyses studied have been prepared by many different workers and are not all equally detailed. In some instances the necessary details were obtainable by correspondence and reference to original notes. The most serious lack of detail involves twenty-eight bogs of southeastern Canada where the counts for *Quercus*, *Tilia*, and *Ulmus* have been lumped (Auer, 1930).

(3) The desired information may be concealed by the fact that, in relation to glacial retreat, sedimentation may not have begun at the same time in all otherwise comparable basins. Thus in younger de-

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I wish to thank Professor Emeritus Frederick O. Grover for many valuable suggestions.

posits more than one genus may be represented by pollen in the lowest level, although the genera may have entered the area at different times.

(4) The intervals of sampling are often arbitrary, in some studies as far as a meter apart. This makes it easy to miss the first appearance of a particular pollen, and may exaggerate the number of apparently simultaneous appearances.

(5) The ecological range of species within the genus *Quercus* is considerable, and it is not practicable to distinguish oak pollens specifically. On the basis of published succession charts, tolerance tables, and personal observation within the area, I have assumed that, in general, oak and hickory are less mesophytic than beech. Certainly the most mesophytic of the oaks in the North Central States, *Quercus borealis maxima*, may be succeeded by beech. This oak also extends farther toward the grassland formation than does beech.

(6) The genus *Pinus* likewise includes a wide ecological range of species. In general, however, I have assumed that the genus is indicative of less available moisture than are *Abies* and *Picea*.

To offset in a measure these various difficulties there are (a) the number and distribution of profiles studied and (b) the general consistency of the maps.

Before the maps for the genera are discussed, the following tables will show the number of profiles in which each genus was not represented, or if present, the order of its appearance. The region covered is diverse climatically and edaphically. If, however, the data be divided to include northern Michigan, northeastern Ohio, Pennsylvania, New York, New England, and Canada in one group, and the rest of the area in the other group, the following relationships appear (tables 1 and 2):

TABLE 1. *Northeastern Area (forty-eight profiles).*

	Absent	1st	2nd	3rd	4th	5th
<i>Quercus</i>	1	42	5	0	0	0
<i>Tsuga</i>	2	30	16	0	0	0
<i>Fagus</i>	20	10	9	9	0	0
<i>Carya</i>	32	5	7	1	3	0
<i>Tilia</i>	33	2	7	4	0	2

TABLE 2. *Southwestern Area (sixty-nine profiles).*

	Absent	1st	2nd	3rd	4th	5th
<i>Quercus</i>	3	63	3	0	0	0
<i>Tsuga</i>	31	10	9	11	6	2
<i>Fagus</i>	44	8	7	7	2	1
<i>Carya</i>	19	17	23	8	1	1
<i>Tilia</i>	18	15	12	18	5	1

Unfortunately, for twenty-eight bogs studied by Auer in southeastern Canada and included in these tables, *Quercus* and *Tilia* are listed together, while no mention is made of *Carya*. In spite of this fact, the tables show (a) the very general early movement of

Quercus, (b) the precedence of *Tsuga* and *Fagus* over *Carya* and *Tilia* in the northeastern area and (c) the precedence of *Carya* and *Tilia* over *Tsuga* and *Fagus* in the more continental area to the southwest.

QUERCUS (fig. 1).—Although the map for *Quercus* contains only eighty symbols, yet it gives the general distribution of the 111 bogs studied. In many cases a single symbol serves for more than one bog in a vicinity. Of the four bogs showing no trace of *Quercus*, three are in northern Wisconsin, and one (Bowman, 1931), the farthest north, is near the Moisie River on the north shore of the St. Lawrence (cf. map of *Tsuga*, fig. 2).

In eight scattered locations, *Quercus* is second in order, being preceded by *Tsuga* in Quebec, Connecticut, Pennsylvania, and Michigan; by *Fagus* in Massachusetts; by *Carya* in Indiana, Michigan and Virginia. In all other instances—105 profiles—it is first in order, with or without other of the five genera accompanying it.

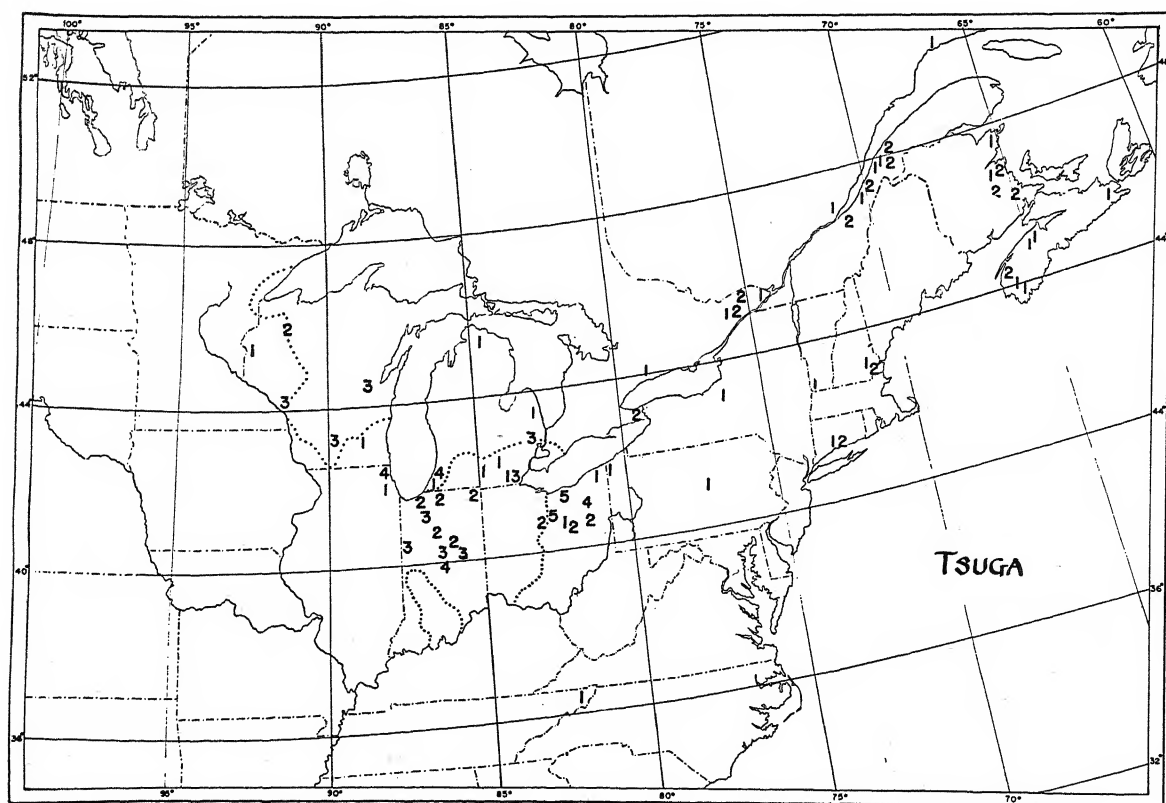
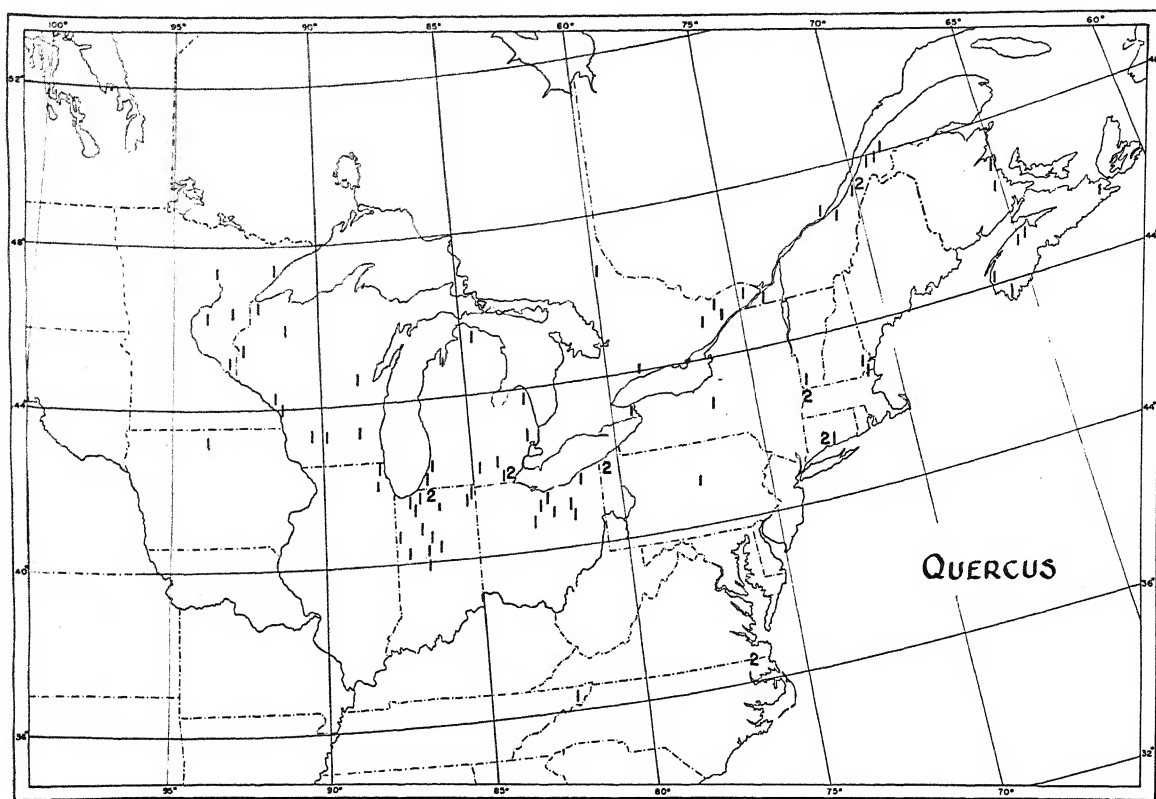
In Ohio deciduous pollen including that of *Quercus* is generally preceded by pollen of *Abies* and *Picea* (Period I), with interesting exceptions as at the glacial margin near Canton (Sears, 1942). Likewise in Indiana, *Abies* and *Picea* generally precede *Quercus* (Prettyman, 1937; Smith, 1937; Otto, 1938, et al.).

However, in the group of bogs in Illinois, Wisconsin and Minnesota described by Voss (1934), oak is present with these conifers and with *Pinus* at the lowest levels. It is also present, with *Pinus* in the lowest levels of the southeastern Canadian bogs described by Auer (1930). Auer, for other reasons, considers these latter bogs to have been initiated in a relatively dry period. Being farther north than those of Ohio and Indiana, they are younger.

Throughout the entire area, as *Abies* and *Picea* are replaced by *Pinus*, there is a noticeable increase in *Quercus*. Thus the general early diffusion of *Quercus* took place during Period II, except in the states bordering the continental grassland, where a suitable continental climate may have prevailed earlier. If my interpretation is correct, Period II and the later Period IV involved eastward extensions of these continental climatic conditions.

The supposed dryness of Period II may have been due to a rise in temperature rather than to a decrease in precipitation. Such a rise in temperature is to be inferred from the known northward retreat of *Abies* and *Picea*.

TSUGA (fig. 2).—The following facts are to be noted from the map of this species. (a) Its earliest appearances tend to be along the Atlantic coast, the St. Lawrence Valley, and in the region of the Great Lakes, including Wisconsin. (b) A number of its later appearances are within those parts of the "prairie peninsula" of Transeau (1935), where it is not now found. Compare the present western limits of its range, as indicated by broken line. (c) In numerous instances it comes in after oak in the St. Lawrence Valley.



Together with the other four genera studied, *Tsuga* is found at the bottom of the profile in eastern Tennessee, consistent with the general belief that this was a region of refuge during glaciation.

A composite pollen spectrum of two samples of peat from the Brooklyn Navy Yard, said to be part of an extensive bed or beds in the Long Island Region, is shown in figure 3 (Sears, 1941b). The spec-

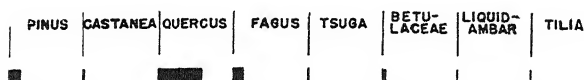


Fig. 3. Pollen analysis of two samples of submerged peat from the Brooklyn Navy Yard, showing relative percentages of various forest trees. *Alnus* and *Betula* are included together.

trum of this peat shows pollen of pine and hemlock, accompanied by oak and other deciduous genera.

This peat is said to be underlain by (boulder?) clay which rests on bed rock and is overlain by outwash gravel. It is about 60 feet below the present surface of Long Island Sound, but, relative to sea level, stood about 100 feet higher during the last glaciation than it does today (Antevs, 1928). This would have placed the surface on which the peat lies at least 40 feet above water. Antevs (1928, p. 90) states that the postglacial submergence "was a very slow process going on until a few thousand years ago." This gives some reason for supposing that the peat is not interglacial. Furthermore, the peat, while compressed, is relatively fresher than most interglacial peat I have examined, again suggesting a postglacial origin.

Whatever the age of this deposit, it is clear that any portion of the continental shelf exposed at the beginning of postglacial time was then available for the northward movement of vegetation. Antevs (1928, p. 91) believes it possible that "part of the now submerged coastal plain may still have been land when the temperature had risen higher than that now prevailing."

If the continental shelf were still above water during Period II, and if warming were chiefly responsible for the relative dryness of this period, both *Tsuga* and *Quercus* may well have moved northward on it during that time. This would apply not only to any coastal route, but equally to interior routes near enough the ocean not to be unfavorable to *Tsuga*.

Furthermore, the increase of *Tsuga* in southeastern Canada should have been definitely favored by the subsequent, warm and more humid Period III which I believe to have existed. This accords with the views of Auer (1930), who comments on the similarity of behavior between *Tsuga* and the deciduous trees. Even in Canada, eleven of the twenty-eight profiles containing both *Tsuga* and *Quercus* show *Tsuga* following *Quercus*.

On the other hand, it is clear from the map that while *Tsuga* either accompanies or follows *Quercus*

in the St. Lawrence Valley and Nova Scotia, it is relatively later in Indiana. The assumed dryness of Period II would certainly have delayed any movement of this genus toward the continental interior. Moreover the supposedly humid Period III offers a reasonable explanation of the relatively late appearance of *Tsuga* in and near those parts of the present prairie peninsula in Ohio and Indiana where it is not now found. The map affords no basis for believing that, in this region, *Tsuga* persisted *in situ* from an earlier, purely coniferous phase, since its pollen is not reported from lower levels. Its appearance here seems to have been merely an intrusion during a favorable mesophytic interlude, followed by withdrawal under less favorable conditions later. It is to be noted that *Tsuga* still persists in southwestern Indiana and in Kentucky, but no pollen profiles are available from this important direction.

Several early occurrences of *Tsuga* are also to be noted, a little beyond its present range limits in Wisconsin, Illinois, and Michigan. In these cases *Tsuga* is definitely associated with the early conifers. These facts suggest an early center of diffusion within the Wisconsin Driftless Area, doubtless a refuge during glacial times and still partly within the range of *Tsuga*.

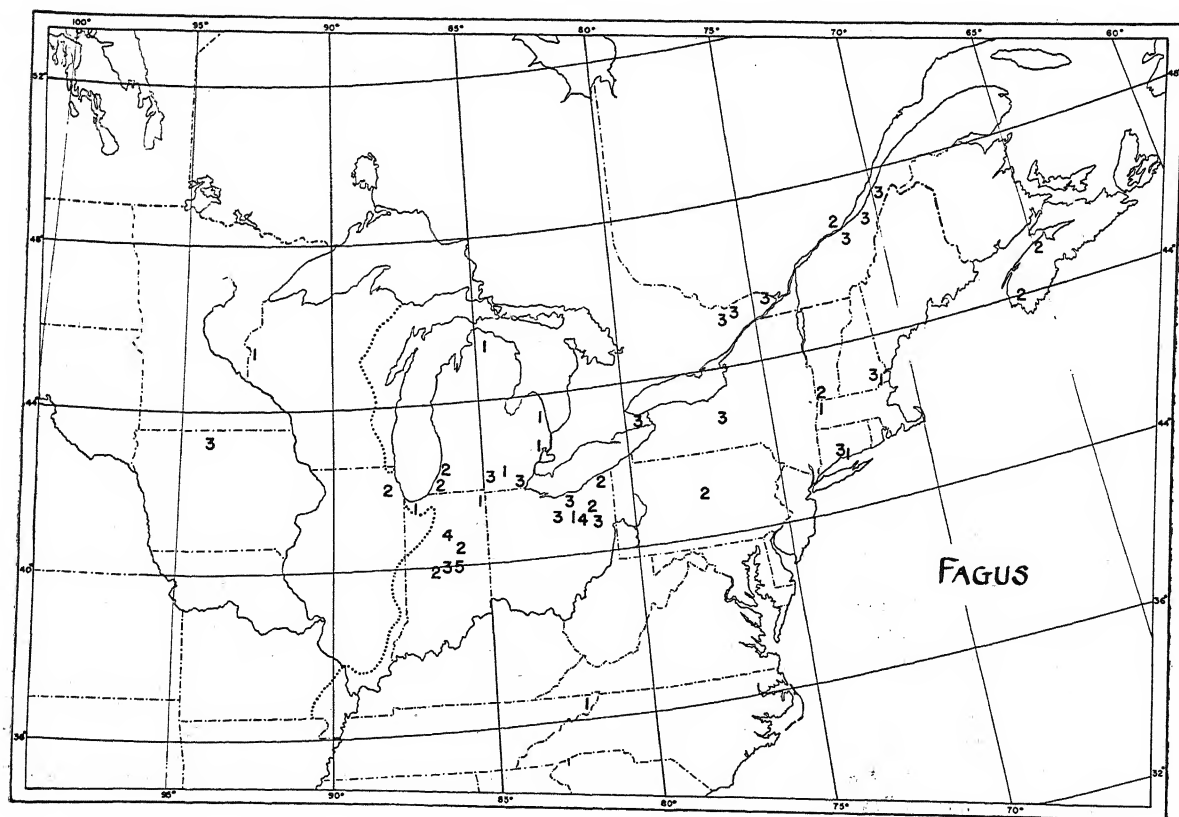
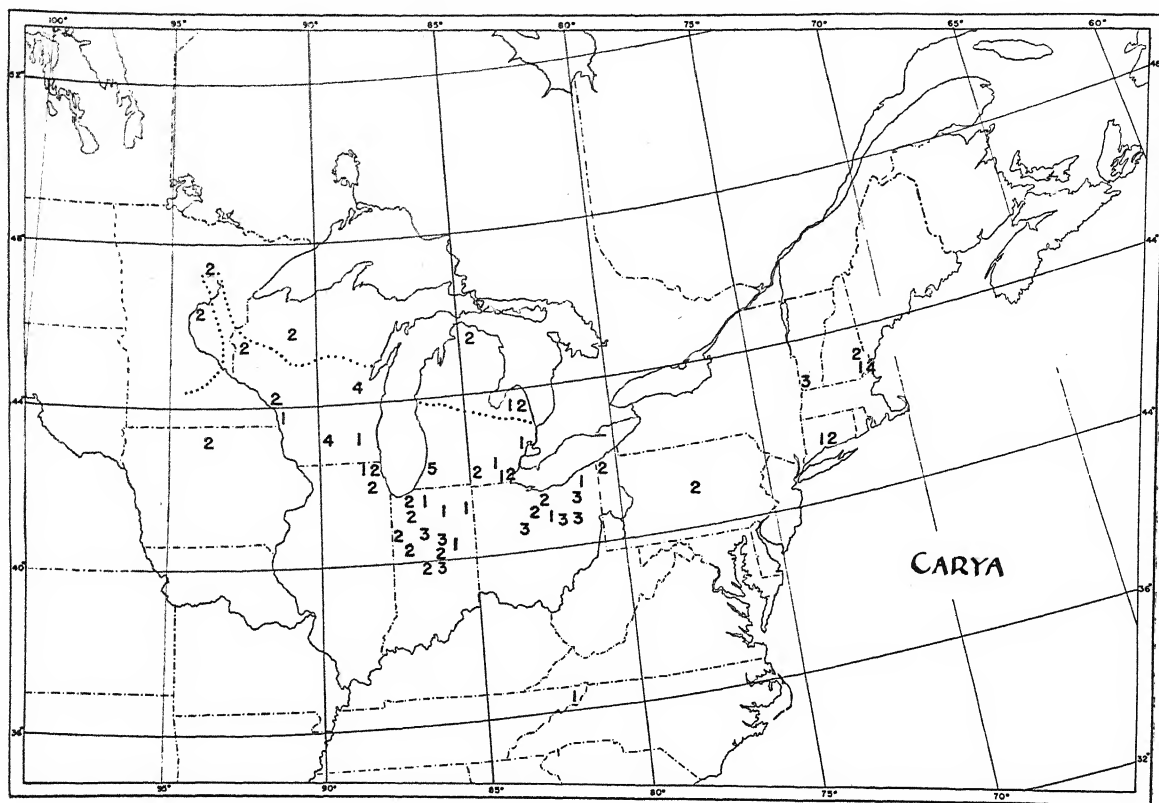
CARYA (fig. 4).—Information regarding this genus is lacking for southeastern Canada. Elsewhere it is relatively early; fifty-two of its sixty-six appearances are either first or second in the series. It is at the first level in Tennessee, Virginia, Connecticut, and New Hampshire. This bespeaks its migration northward along the Atlantic coast. It is also present in the submerged Long Island peat.

Elsewhere its early occurrences are at the periphery of the prairie peninsula and in the Wisconsin Driftless Area and re-entrant. Conceivably it may have moved northwest into the Great Lakes region along routes not revealed by the present study. Data from the lower Ohio Valley are needed and a most detailed study of sediments in and near the Driftless Area should be made.

Profiles from the North Central States rather frequently show *Carya* present with *Quercus* during Period II. This was followed by a relative decrease during Period III with a subsequent maximum during Period IV.

Most interesting, however, is its behavior with reference to the present northern range limit of the genus, as shown by a broken line based upon data from Messrs. Darlington, Fassett, Goodman, and Lawrence. *Carya* pollen occurs in four profiles north of its present range, and in another profile in Itasca County, Minnesota, where its modern occurrence is sporadic. In all of these five profiles its behavior is identical. It shows as a trace, and only at the level of the distinct oak maximum which marks Period IV. This oak maximum is characteristic of many bogs in the southern part of the present coniferous region (Sears, 1935) and corresponds to the only ap-

Fig. 1-2.—Fig. 1 (above). Map showing relative order of appearance of *Quercus*.—Fig. 2 (below). Map showing relative order of appearance of *Tsuga*.



pearance of oak (q.v.) in the bog north of Georgian Bay (Janson and Halfert, 1937).

The apparent northward advance and subsequent retreat of *Carya* thus supports the idea that Period IV was a time of maximum warmth during postglacial time. Farther south the decrease of *Fagus* and *Tsuga* during Period IV and the accompanying increase of *Quercus* and *Carya* afford a basis for believing that it was also relatively drier than Period III.

These circumstances, together with abundant direct evidence from profiles in the North Central States and southeastern Canada, indicate strongly that the range of *Fagus* expanded during Period III and retracted during the subsequent drier Period IV.

Pending the necessary critical studies, the suggestion is offered that *Fagus* had a glacial refuge in the Driftless Area of Wisconsin as well as one in Tennessee. Data from the lower Ohio and Missouri Valleys should be of great interest.

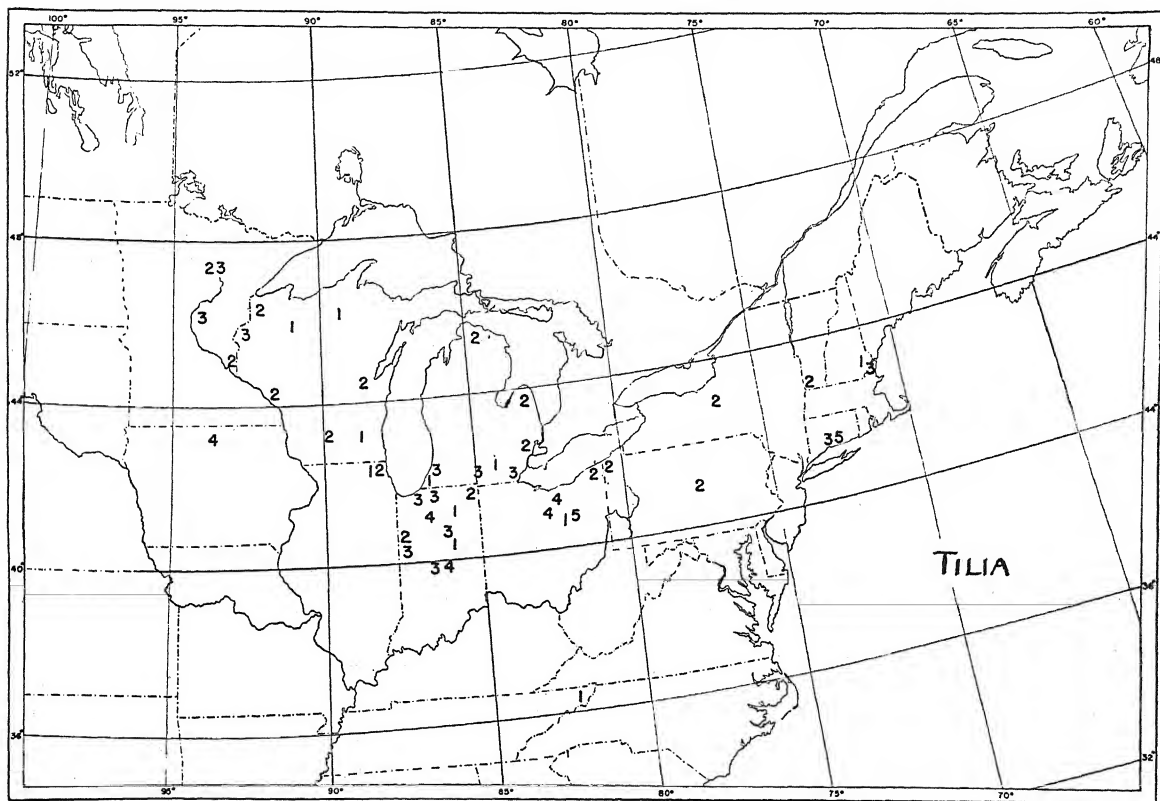


Fig. 6. Map showing relative order of appearance of *Tilia*.

Fagus (fig. 5).—*Fagus* appears first in order along the Atlantic coast, in Connecticut, Massachusetts and New Hampshire. It obviously follows *Quercus* and *Tsuga* in the St. Lawrence Valley. Other early occurrences were in the western portion of the area at the edge of the prairie peninsula, near the Driftless Area and over the southern peninsula of Michigan. The apparent movement of beech into central Indiana resembles that already described for *Tsuga*.

Especially notable are three records for *Fagus* in Iowa, Wisconsin, and Illinois, respectively, which clearly lie to the west of the present critical limits of this genus as outlined by Transeau (1935) and shown here by a dotted line.

Fagus is specialized, requiring humid conditions and occupying a late stage in forest succession.

TILIA (fig. 6).—Unlike *Carya*, *Tsuga*, and *Fagus*, this genus does not appear in any profiles outside of its present range. It shows relatively few early stations, namely one in New Hampshire, several at the periphery of the prairie peninsula, and two, very definitely, in northern Wisconsin. Its movement westward from Wisconsin into Minnesota and Iowa, where it followed *Fagus*, seems clearly indicated.

Whether it entered from a southwestern refuge, or persisted in the Wisconsin re-entrant, or both, its early appearances in Wisconsin, Illinois, Indiana and Michigan represent its position during Period II. Its movement to the west and southwest was subsequent, apparently favored by the more humid Period III, as were *Fagus* and *Tsuga*. But unlike them, it was able to hold its ground and even to advance beyond the Missouri River, where today, with

Fig. 4-5.—Fig. 4 (above). Map showing relative order of appearance of *Carya* (add 1 at location of Dismal Swamp, Virginia).—Fig. 5 (below). Map showing relative order of appearance of *Fagus*.

Quercus borealis maxima, it represents the most mesophytic stage of forest succession (Pool, Weaver and Jean, 1918).

SUMMARY

The relative order of appearance of pollen of *Quercus*, *Tsuga*, *Carya*, *Fagus*, and *Tilia* in 111 peat deposits within an area extending from Iowa to the Atlantic, and from Tennessee to the St. Lawrence is here reported. Resultant inferences concerning order, direction, and extent of migration are discussed in relation to supposed changes in postglacial climate.

The general order of appearance in the northeastern part of the area was apparently: *Quercus*, *Tsuga*, *Fagus*, *Carya*, and *Tilia*; in the southwestern part: *Quercus*, *Carya*, *Tilia*, *Tsuga*, and *Fagus*.

The early and general dispersal of *Quercus* seems to have occurred when *Pinus* replaced *Abies* and *Picea*. This shift in composition coincides with a supposed dry period, possibly resulting from rising temperature rather than from decreased precipitation. *Tsuga* appears to have accompanied *Quercus* into eastern Canada at this time in the relatively moister climate of the Atlantic region. In the interior, *Quercus* was frequently accompanied by *Carya*.

Later the ranges of *Tsuga* and *Fagus* apparently expanded, not only within southeastern Canada, but farther west into the area beyond the present limits of these genera. This is regarded as an indication that climate in the continental interior was then more humid than it had been just previously. *Tilia* also seems to have moved westward, during this more humid time, toward its present limits.

After that, *Carya* evidently extended north of its present general limits. This extension is definitely correlated with a decrease in *Tsuga* and *Fagus* and with a maximum of *Quercus* in the coniferous bogs at the northern edge of the area. It is regarded as indicating a second dry and warm period, now past its climax.

Important early centers of dispersal appear to have existed in the southeastern part of the area and in Wisconsin, a considerable part of which was not covered by the last glaciation.

This does not preclude the existence of other early dispersion centers and routes which may be revealed when the necessary intensive local studies are made. Data from the lower Ohio Valley should be of especial importance.

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PACIFIC SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PACIFIC SECTION OF THE BOTANICAL SOCIETY OF AMERICA, SALT LAKE CITY, UTAH, JUNE 15 TO 18, 1942

SOME DESMIDS OF UTAH. *Seville Flowers, University of Utah, Salt Lake City, Utah.*—The present account of Utah desmids is a list of species collected over a period of twenty years together with data and notes citing previous records. The principal localities in which they were collected center around the lakes in the Uintah Mountains and Wasatch Mountains. A few other localities are included. At present about one hundred twenty-five species and varieties are sufficiently well known to be included in this list. Others are still lacking definite confirmation and may be published later.

The following is an enumeration of the genera and species to be cited in the complete paper: *Arthrodesmus*, five species; *Calocylindrus*, two species; *Closterium*, nineteen species; *Cosmarium*, thirty-two species; *Euastrum*, thirteen species; *Desmidium*, two species; *Gymnozyga*, two species; *Hyalotheca*, one species; *Micrasterias*, fourteen species and varieties; *Netrium*, three species and varieties; *Pleurotaenium*, three species; *Sphaerosozma*, two species; *Spirotaenia*, one species; *Stauroastrum*, twenty-one species and varieties; *Xanthidium*, seven species and varieties.

FURTHER STUDIES ON THE SAPROLEGNACEAE OF SOUTHERN CALIFORNIA. *James V. Harvey, San Bernardino Valley Junior College, San Bernardino, California.*—Central and northern California stations have offered a few species of the true water-molds: *Saprolegnia delicata*, once; *Saprolegnia* sp. (non-sexual form, with oddly-shaped gemmae), once; *Achlya pinnulata*, n. sp., three times; *Achlya* sp., once; *Aphanomyces* sp. (non-sexual), once; and *Dictyuchus sterile*, five times.

The following species appear to be endemic to localized areas in the San Bernardino Mountains or adjacent valleys in southern California: *Saprolegnia bernardensis*, n. sp., six times; *Saprolegnia delicata*, five times; *Saprolegnia* sp., six times; *Achlya caroliniana*, eight times, and *A. diffusa* n. sp., four times.

Achlya heteromorpha n. sp., female strain, has been taken twenty-nine times, mostly in and near the city of San Bernardino. It was obtained once in Death Valley, along with *Saprolegnia ferax*, *Dictyuchus sterile*, and *Aphanomyces* (a non-sexual form).

Achlya heteromorpha n. sp., male strain, was taken twice, at stations 200 miles apart, in southern California.

Dictyuchus sterile has been most abundant of all species distributed uniformly over all of California, eighty-nine times. A sexual strain of *Dictyuchus* has been isolated five times.

Other species occurring plentifully in the entire state include: *Saprolegnia ferax*, seventy-five times; *Saprolegnia delicata*, seventeen times; *Saprolegnia* sp. (non-sexual), two distinct forms described as new, eighty-six and seven times respectively; other species of *Saprolegnia*, unidentified, fourteen times; *Achlya pacifica*, n. sp., nine times; *A. pinnulata*, n. sp., five times; *A. californica*, n. sp., ten times; *A. sp.*, nineteen times, nine of these producing no sexual bodies; and *Aphanomyces* (a non-sexual strain), fifty-eight times.

A few species, less plentiful, include: *Aphanomyces balboensis*, n. sp.; *A. scaber*; *A. laevis*; *A. sp.*; *Leptolegnia* sp. (non-sexual); *Geolegnia inflata*; *G. septisporangia*; *Brevilegnia megasperma*; and *Calyptralegnia unisperma* var. *litoralis*. Species of *Olpidiopsis* (parasitic) have been taken three times.

RELATIONSHIP OF TOP GROWTH TO SUGAR BEET CROWN TEMPERATURES AND INDUCTION OF FLOWERING. *Eubanks Carsner and Bion Tolman, United States Department of Agriculture, Riverside, California.*—Initiation of the seed-stalk and flowering in sugar beets is brought about mainly by the cumulative effect of prolonged low temperature exposure followed or accompanied by the effect of long photoperiods. In a seed growing area it is impossible to control air temperatures during the overwintering period, but it is possible to modify soil temperatures and in a measure control the thermal environment of

the beet crowns. Modification of soil and crown temperatures is brought about mainly by control of foliar growth. Foliar growth is controlled by planting date, fertilizer practices and spacing of plants. In southern Utah, the development and maintenance of an extensive growth of leaves to shade the soil and help create the most effective temperature range for thermal induction proved to be beneficial.

THE RELATION OF TEMPERATURE TO REPRODUCTION IN SUGAR BEETS. *Myron Stout, United States Department of Agriculture, Riverside, California.*—The economic varieties of sugar beets are biennial plants which require the influence of cool temperatures, combined with or followed by long photoperiods to induce reproductive development. The relative extent of thermal induction during storage at different temperatures was measured by conducting bolting tests in a warm environment under long photoperiods. The beets bolted more rapidly and completely after storage at 6° to 8°C. than after storage at lower temperatures. There was little increase in the bolting tendency of beets as taken from the field in mid-winter, after storage at temperatures near 0°C. The bolting tendency of beets, which were taken from the field in mid-winter, was greatly reduced by a comparatively short period of storage at a temperature of 23° to 24°C. This indicates that the biochemical processes associated with thermal induction are reversible and that the rate of change is greatly influenced by temperature.

MALE STERILITY IN SUGAR BEETS PRODUCED BY COMPLEMENTARY EFFECTS OF CYTOPLASMIC AND MENDELIAN INHERITANCE. *F. V. Owen, United States Department of Agriculture, Riverside, California.*—The nature of the inheritance of male sterility in beets indicates two types of cytoplasm. Plants with N cytoplasm produce normal and abundant pollen. Plants with S cytoplasm may produce aborted pollen and become completely male sterile. The breeding evidence indicates that several Mendelian factors may influence pollen development when carried by plants with S cytoplasm, but the same factors have no effect when carried by plants with N cytoplasm. Most of the breeding behavior is explained by assuming that two Mendelian factors, *x* and *y*, have effects complementary to the influence of the S cytoplasm as follows:

$S\ x\ x\ z\ z$	Male sterile with white, empty anthers.
$S\ X\ x\ z\ z$	
$S\ x\ x\ Z\ z$	Semi-male sterile with yellow anthers but
$S\ X\ X\ z\ z$	little or no viable pollen.
$S\ x\ x\ Z\ Z$	
$S\ X\ x\ Z\ z$	More or less normal pollen depending upon
$S\ X\ X\ Z\ z$	favorable or unfavorable environmental
$S\ X\ x\ Z\ Z$	conditions, but the anthers sometimes fail
$S\ X\ X\ Z\ Z$	to open.

Crosses between male sterile ($S\ x\ x\ z\ z$) ♀ and normal ($N\ x\ x\ z\ z$) ♂ plants produced F_1 offspring, all of which were completely male sterile. Crosses between semi-male sterile ($S\ X\ x\ z\ z$) ♀ and normal ($N\ x\ x\ z\ z$) ♂ plants produced F_1 offspring,

approximately half of which were male sterile ($S\ x\ x\ z\ z$) and half semi-male sterile ($S\ X\ x\ z\ z$). Reciprocal combinations ($N\ x\ x\ z\ z\ \varnothing \times S\ X\ x\ z\ z\ \sigma$) sometimes made possible by the scant production of viable pollen on $S\ X\ x\ z\ z$ plants, produced F_1 offspring all of which bore completely normal and abundant pollen.

The unique nature of the inheritance of male sterility in sugar beets has facilitated the wholesale emasculation of plants for hybridization purposes.

COMPARATIVE INFLUENCE OF CLIMATE AND GRAZING ON RANGE PLANT COVER. *Selar S. Hutchings, Intermountain Forest and Range Experiment Station, Ogden, Utah.*—The comparative influence of climatic factors and those of grazing on plant cover and plant succession constitutes one of the most controversial questions in range management. Some individuals^{1,2} maintain that range deterioration and changes in plant cover result largely from fluctuations in climate. Others³ believe that climatic factors exert a marked influence on forage yields and plant succession but in the main they do not result in wholesale replacement of plant species. Comparisons of vegetation at eight locations in 1934, following a period of severe drought, and again in 1941, during a period of good precipitation, indicate that plant reaction to drought and precipitation results primarily in changes in production and loss of less tolerant plants. These may not be the more palatable plants, since shadscale and curlygrass, species of low palatability, suffered most from drought. Reduction in plant cover from overgrazing results in a differential loss of the more palatable forage plants. Heavy grazing reduces the plant vigor, thins the plant cover, prevents seedling reproduction, and makes possible the invasion of species low in palatability, including introduced annuals such as Russian thistle, which in some cases completely replaces native forage plants.

INFLUENCE OF TIME AND FREQUENCY OF GRAZING ON RANGE READINESS AND FORAGE PRODUCTION. *George Stewart, Intermountain Forest and Range Experiment Station, Ogden, Utah.*—Ranges on which grazing began early in spring, that is, soon after plant growth had started, have almost invariably deteriorated. Range administrators, therefore, deemed it necessary to prevent this early grazing which they came to designate as "premature" grazing. As more research was done, however, the causes and effects were found by no means to be as simple as the original evidence and conclusions seemed to warrant. In many cases early grazing

¹ American National Live Stock Assn. If and when it rains; the stockman's view of the range question. Denver, Colo. 1938. 57 pp., illus.

² Alter, J. Cecil, and Ralf R. Wolley. Precipitation and vegetation. Presented at the November, 1937, Utah Academy of Science meetings.

³ Stewart, George, W. P. Cottam, and Selar S. Hutchings. Influence of unrestricted grazing on northern salt desert plant associations in western Utah. Jour. Agric. Res. v. 60(5): 289-316, illus. 1940.

merely permitted another grazing to follow after a short interval. Frequency of grazing has proved in the long run to be a more potent cause of plant weakening than early grazing *per se*.

Early grazing is damaging, however, at least on mountainous ranges, since the ground is soft in early spring, and tramping by animals, therefore, tends to uproot plants, especially seedlings. The grazing of seedlings is in itself likely to be destructive to forage reproduction either by uprooting the young plants or by removing the shoots and leaves before the plant has become self-supporting. Early grazing does minor injury to established perennials provided they are not again grazed until maturity. Regrowth after early grazing is slow in cool weather, and repeated grazing reduces storage of food-reserves, thereby weakening the plants. Forage during its early growth is chemically rich but low in total yield, which may make the grazing capacity small for some weeks.

SOME EDAPHIC AND CLIMATIC RELATIONSHIPS IN PLANT SUCCESSION AND HABITAT BALANCE. L. A. Stoddart and A. D. Smith, *Utah State Agricultural College, Logan, Utah*.—Soil forming processes acting upon rock materials make, in early development, an increasingly productive soil in terms of quantity and, also diversity of plant species produced. Productivity may increase until the soil and its biome reach a point of relative stability with the climate. The nature and, hence, productivity of soil at this "climax" point is determined by climate. The parallelism and interdependence existing between soil development and normal plant succession is of great importance to the ecologist, although too often it is overlooked. Soil plays a varying rôle as a determinant or limiting factor to plant production—both volume and species composition. In this rôle it alternates somewhat with climate as succession progresses or regresses. Different climates induce different relationships.

Through successional development, the biome and the soil attain a balance with the climate as marked by a virtual cessation of trends. The biotic and edaphic "climax" can be said to have been reached. Further changes are oscillatory or cyclic and result from fluctuating weather conditions and temporary predominance of competing members of the climax vegetation. Apparently, either the soil or the climate may be the direct factor determining the point of relative stability of vegetation.

POST-PLEISTOCENE VEGETATION AND CLIMATE OF THE PACIFIC NORTHWEST. Henry P. Hansen, *Oregon State College, Corvallis, Oregon*.—A preliminary study of post-Pleistocene forest succession in the Pacific Northwest has been made by pollen analyses of peat bogs and lake sediments. In the Puget Lowland of Washington, the climax forests are composed of western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*), with Douglas fir (*Pseudotsuga taxifolia*) persisting in predominance as a result of recurring fire. The pioneer postglacial

forests consisted chiefly of lodgepole pine (*Pinus contorta*), which was replaced by Douglas fir, and which in turn was partially replaced by western hemlock.

On the Oregon Coast, the climax forest consists of western hemlock and Sitka spruce (*Picea sitchensis*). The post-Pleistocene forests have consisted of these species in about equal abundance. In the Willamette Valley of Oregon, the principal tree species are Douglas fir and Oregon white oak (*Quercus garryana*). The initial post-Pleistocene forests were composed of lodgepole pine, Sitka spruce, and lowland white fir (*Abies grandis*). These were slowly replaced by Douglas fir, which in turn was replaced by oak in comparatively recent time. On the east slope of the central Cascades of Oregon, the climax forests are composed of lodgepole and western yellow pine (*Pinus ponderosa*). The former persists as an edaphic climax because of an extensive pumice mantle deposited by post-Pleistocene volcanic eruptions. One of these was the eruption of Mount Mazama that formed Crater Lake, about 5,000 years ago. The pollen record of bogs in this region shows several trends toward yellow pine predominance, which were interrupted by the deposition of pumice, but lodgepole apparently has been predominant since the first major eruption.

In northern Idaho the climax forests consist of western hemlock, western red cedar, and lowland white fir, with western white pine (*Pinus monticola*) persisting as a subclimax due to recurring fire. The pioneer post-glacial forests consisted principally of lodgepole and white pine. The former was superseded by white pine, which remained predominant during the rest of the post-Pleistocene. A definite trend toward a climax forest occurred in recent time, but it was apparently interrupted by fire. In northeastern Washington, the climax forest is composed largely of yellow pine. The pioneer post-glacial forests consisted of lodgepole and white pine, and western larch (*Larix occidentalis*). These were succeeded by chenopods, grasses, and composites, suggesting a hot, dry period. These were slowly replaced by yellow pine, which has persisted to the present.

In north central Washington, the climax forests are composed of yellow pine, Douglas fir, and western larch. The initial post-glacial forests were composed of lodgepole, white, and yellow pine, with the first predominant. Yellow pine gradually assumed predominance during the post-Pleistocene, with some Douglas fir in more recent time. East central Washington is covered with the bunchgrass prairie and sagebrush and its associates. A peat profile in this region shows that grass has been predominant during most of the post-Pleistocene. Trees recorded in significant proportions are lodgepole, white, and yellow pine, although the nearest present-day forests are thirty miles away. Chenopods and composites are also recorded abundantly throughout. The post-Pleistocene general climatic trend has apparently been one of warming and drying to a maxi-

mum, followed by cooling and a slight increase in moisture to a degree which has persisted to the present.

EFFECT OF NUTRITION ON VACUOLAR COMPONENTS. *Howard S. Reed, University of California, Berkeley, California.*—Cellular inclusions have for over a century received attention from plant cytologists, and they have generally agreed that colloids of one sort or another are present in plant cell vacuoles. Recent studies show that these materials have great significance in the activity of the cell and are intimately related to health and disease. A characteristic type of spherical inclusion, consisting of catechol, has formerly been mistaken for micro-organisms in the cell. The present work describes how these bodies are formed in the vacuolar solution by a separation of phases. These globules consist of catechol enveloped in a layer of phospholipoid. The coacervated aggregates have been found in the vacuoles of hypoplastic cells of leaves, in the post-meristematic cells of growing shoots of apricot trees suffering from little-leaf, as well as in the roots of orange seedlings growing in zinc-deficient nutrient solutions. The globules of this type have never been observed in the cells of healthy plants.

THE ZINC CONTENT OF "LITTLE-LEAF" AND NORMAL LEAVES. *Elmer Archibald and F. B. Wann, Utah State Agricultural College, Logan, Utah.*—In the State of Utah a serious "little-leaf" condition has been observed on fruit trees in local areas of Utah, Davis, and Boxelder Counties. Since the application of zinc to the plants corrects the condition, it is probable that the "little-leaf" is due to a zinc deficiency. Zinc sulphate applied as a foliage spray has given good control. Chemical analyses for zinc in diseased and normal leaves of peach, cherry, apple, and plum showed: (a) little or no transverse movement of zinc in the plant; (b) higher zinc content in leaves from trees sprayed with zinc sulphate than in leaves from trees treated by other control methods. Addition of zinc to the soil did not increase the zinc content of leaves. (c) Trees with less than 123 micrograms of zinc per gram of dry matter showed little-leaf symptoms; healthy leaves contained from 123 to 345 micrograms of zinc per gram of dry matter.

WHICH FACTORS LIMIT THE GROWTH OF TOMATO PLANTS? *F. W. Went, California Institute of Technology, Pasadena.*—By growing tomatoes in air-conditioned greenhouses reproducible results on tomato growth were obtained.

So far it has not been possible to increase the growth rate of plants 50–100 cm. tall beyond 30 mm./day. At that rate none of the known plant hormones limit growth. Growth can be increased neither by a change in the nutrient solution, nor by increased aeration of the roots, nor by higher temperatures, nor by more light, nor by different photoperiods, nor by other air humidities nor by changed feeding schedules. Under sub-optimal conditions the water supply or the nutrient supply or the wrong photoperiod or auxin or the caulocaline production can

each be limiting. It was found, however, that under optimal conditions the sugar supply towards the growing point is limiting. Experiments under different temperature conditions suggest that it is the sugar transport rather than photosynthesis which is the bottleneck in tomato stem growth, just as it seems to be for fruit growth.

THE ORIGIN OF THE WESTERN SPECIES OF DRABA. *C. Leo Hitchcock, University of Washington, Seattle, Washington.*—*Draba*, a genus of the Mustard family, is predominantly a plant of arctic or montane habitat. Several of the perennial species apparently moved southward in western North America with the invasion of the ice sheets and were quite widespread in range at one time. At the present time most of these species are "stranded" on the higher peaks of the Rocky, Cascade, and Sierra Nevada Mountains.

Subsequent to the ice ages, variation in these and isolated populations has resulted, in many instances, in the formation of several closely related, but genetically different specific, or subspecific entities. In some species, however, no such noticeable variation has occurred, relict populations of circumpolar species persisting as far south as Colorado.

The discussion will be concerned with the possible origin of most of the montane species of the genus as it occurs in western United States.

ORIGIN AND AFFINITIES OF THE FLORA OF CENTRAL ROCKY MOUNTAINS. *Joseph Ewan, University of Colorado, Boulder, Colorado.*—Five sources may be recognized for the region: (a) *holarctic*, the largest and most prominent element, of which the boreal floras are almost entirely composed, and which even extends down and outwards from cordillera to plains adjacent; (b) *southeastern*, small but distinctive group, remotely of Mexican origin but entering our region through the lower Mississippi Valley; (c) *southwestern*, second largest element, remotely of Mexican origin but by way of (?) secondary centers most immediately related to present Arizona-New Mexican floras; (d) *Pacific*, small but characteristic element, possibly entering via the Columbia River catchment basin; (e) *endemic*, weak group, the components related either to holarctic or southwestern elements. Criteria for determination of the past movements of any given group are: (a) present greatest concentrations of species; (b) comparative morphology of peripheral members of the group against that of members of concentration centers; (c) distribution of endemic species of the group with relation to its periphery; (d) comparative data from cytogenetics (where available) as to derivative chromosomal types with reference to parental diploid species. The fact that all groups of vascular plants from Pteridophyta to the most highly evolved Sympetalae exhibit this past migration and have participated in it lends support to the belief that there must have been pre-Pleistocene movements of this flora.

TAXONOMIC-ECOLOGICAL RELATIONSHIPS OF THE GENUS ZIGADENUS. *O. S. Walsh, Montana State College, Bozeman, Montana.*—The spelling *Zigadenus*

is adopted to conform to the original spelling used by Michaux when the generic name was first published.

The species of *Zigadenus* are highly polymorphic, exhibiting many growth forms as expressions of adaptability to environmental conditions. Kind and availability of soil moisture are apparently the major factors controlling distribution and causing greatest variation in growth habit in many species.

Experimental plants confirmed certain observed growth habit tendencies as environmental adaptations common to all species. Clusters of plants, often large and with numerous sheathing basal leaves and some suffrutescence, characterize growth in moist areas. Slender, sparse plants widely separated occur mostly on rocky or dry areas. Alkalinity reduces height growth but induces larger or heavier succulent foliar growth and large bulbs. Progressive dwarfing appears in all montane species near the upper limits of their altitudinal range, and modifies other growth tendencies. Many previously named "species" have been based on these habitat variations.

Proposal is made to retain sixteen species as valid and reduce four additional "species" to sub-specific rank. Most other previously named species are considered to be "formae" or environmental adaptations only. Names for these habitat forms are not recommended.

NOTEWORTHY PLANTS OF NORTHEASTERN NEVADA. A. H. Holmgren, *Utah State Agricultural College, Logan*.—A study of the flora of northeastern Nevada has resulted in several interesting discoveries. The following list includes new records for the state, and range extensions within the state. The asterisk denotes which are apparently hitherto unreported for the state.

Botrychium Lunaria (L.) Swartz var. *minganense* (Vieill.) Dole; **Selaginella selaginoides* (L.) Link; *Najas marina* L.; **Triglochin palustris* L.; **Lilaea subulata* H. B. K.; **Damasonium californicum* Torr.; **Glyceria occidentalis* (Piper) J. C. Nels.; **Poa arctica* R. Br.; **Eragrostis hypnoides* (Lam.) B. S. P.; **Triodia pilosa* (Buckl.) Merr.; **Blepharidachne Kingii* (S. Wats.) Hack.; **Agrostis humilis* Vasey; **Hierochloa odorata* (L.) Beauv.; **Carex brunnescens* (Pers.) Poir.; **Carex filifolia* Nutt.; **Carex limosa* L.; **Juncus articulatus* L.; **Luzula spicata* Desv.; **Corallorrhiza striata* Lindl.; *Salix Lemmoni* Bebb; *Eriogonum chrysocephalum* A. Gray subsp. *desertorum* Maguire; *Halogeton glomeratus* (Bieb.) Mey.; *Arenaria Franklinii* Dougl.; **Nymphaea odorata* Ait.; **Ranunculus testiculatus* Crantz; *Aquilegia caerulea* James; *Lepidium nanum* S. Wats.; *Draba Douglasii* A. Gray; *Trifolium monanthum* A. Gray; *Linum Kingii* S. Wats. var. *sedoides* Porter; **Viola bellidifolia* Greene subsp. *valida* Baker; **Epilobium lineare* Muhl.; **Eryn-*

gium alismaefolium Greene; **Lomatium montanum* C. & R.; **Gratiola neglecta* Torr.; *Galium Matthewsii* A. Gray; **Aplopappus lanuginosus* A. Gray subsp. *Andersoni* (Rydb.) Hall; *Erigeron Austiniae* Greene; *Erigeron compactus* Blake; *Stylocline micropoides* A. Gray; **Stylocline filaginea* A. Gray; *Wyethia helianthoides* Nutt.; *Eatonella nivea* (D. C.) Eat. A. Gray; *Tanacetum potentilloides* A. Gray.

THREE NATURAL HYBRIDS OF THE COMPOSITAE IN WYETHIA AND BALSAMORHIZA. Bassett Maguire, *Utah State Agricultural College, Logan*.—Three putative natural inter-specific hybrids in *Wyethia* and *Balsamorhiza* are reported. Evidence of field association and of comparative morphological study are offered in support of these propositions.

In numerous areas in Idaho *W. amplexicaulis* Nutt. and *W. helianthoides* Nutt. occur growing intimately together. In such instances approximately 15 per cent of the mixed population show intermediacy of the essential morphological characters. Evidence seems to indicate that these intermediate plants are natural hybrids and are fertile, and that they may back cross with either parent. On no occasion where both species were not present, did plants of intermediate character occur. The putative hybrid *W. amplexicaulis* × *helianthoides* had previously been described as *W. Cusickii* Piper.

A somewhat comparable but more complex situation was observed to take place in *Balsamorhiza* in northeastern Nevada, involving *B. sagittata* as one probable parent, and either or both of two forms which had earlier been interpreted as phases of *B. hirsuta*, as the other probable parent. One of these forms seems to be a minor variant of *B. hispidula*, which entity appears to be no more than a geographical segregate of the more northern *B. hirsuta*. The other, differing from the first in distressingly little beside character of pubescence, is, however, similar to specimens designated by Sharp as *B. platylepis*, plants which seem to be too close to *B. Hookeri* for specific distinction. Both of these forms occur on thin stony soil, sometimes intermixed, while in the same region *B. sagittata* occupies more favorable deeper soil. Where the area of either or both of the two populations becomes contiguous with that of *B. sagittata* invariably morphologically intermediate plants occur in the ecotone between them. These intermediate plants have been designated as putative hybrids between *B. sagittata* and *B. platylepis* (? a ssp. of *B. Hookeri*) on the one hand, and between *B. sagittata* and *B. hispidula*, var. (? a ssp. of *B. hirsuta*) on the other.

Transplants of all forms of both genera concerned were found to be easily made. Attempted germination of seed was not successful. These materials are suggested as offering excellent opportunity for cytogenetical and experimental taxonomic study.

STRUCTURE AND GROWTH OF THE VEGETATIVE SHOOT APEX OF *GARRYA ELLIPTICA* DOUGL.¹

Roger M. Reeve

THE RESEARCHES of Grégoire (1935a and b, 1938) and Louis (1935) have brought attention to a number of the neglected problems of foliar and floral histogenesis. Grégoire (1935b) rejected the classical interpretation of the flower and insisted that no homologies exist between vegetative and floral apices. Louis, in detailed comparative studies of a number of species, has shown the interrelationship of shoot and lateral organs from the earliest stages in ontogeny of the primordia up to and including stages of early vasculature. The significance of these contributions, and the studies of earlier workers, as related to phylogenetic interpretations of the vegetative shoot and the flower, have been reviewed by Foster (1936, 1939, 1941).

Zimmerman (1939) has correlated evolution and ontogeny in a broad concept which he terms "hologeny." In this very interesting theoretical treatment the evolution and the ontogeny of plant organs are projected into a time and space relationship descriptive of the evolution and developmental history of the entire organism. In the writer's opinion, further detailed histological studies conducted in an objective manner can only lend support to such a viewpoint. Unfortunately, many phylogenetic interpretations have been based upon incomplete information on organ histogenesis, and, as yet, the stages of transition from vegetative apices to floral apices have been neglected.

The present study on the apices of *Garrya* was undertaken in an effort to contribute further information related to these problems. The genus was chosen because the different sorts of apices were readily obtainable on the same plants throughout the year near Berkeley, and because the decussate phyllotaxis facilitated interpretation of serial sections. There have been few detailed studies made of the apices of woody, amentiferous species. These observations on the vegetative shoot will be followed with comparative studies on catkin ontogeny.

The author is indebted to Professor A. S. Foster, under whom this study was undertaken, for aid and encouragement during the progress of the research. Appreciation is also accorded to Professor Katherine Esau for kindly criticism and suggestions.

TAXONOMY AND GENERAL ORGANOGRAPHY.—The Garryaceae consists of the single genus *Garrya* and has been variously placed in systems of classification. Prevalent among the ideas of its affinity is either that it is closely related to or is included with the Cornaceae. Baillon (1877a and b) and Hallock (1930) have discussed the opinions of the various systematists and are in general agreement with those who place the Garryaceae in the Umbellales. There

is no intention in this paper to advance further suggestions concerning the phylogeny of the family. It is significant, however, that morphogenetic investigations have been made upon relatively few members of the Umbellales.

Floristically, *Garrya* is an old genus belonging to the Mexican Plateau element. It has been found in the Miocene, and once must have been more widely distributed than at present. Present distribution is local, ranging from Oregon into Central America and the West Indies. In California there are three or four fairly distinct species between which occur intermediate forms strongly suggesting hybridization at a time when the genus was more general in distribution.

The growth habit is more or less uniform throughout the genus, varying from woody, dioecious shrubs to diffusely branched, tree-like forms. The buds are protected by a series of acute cataphylls which are clothed with dense, sclerotic epidermal hairs in most of the California forms. The cataphylls are formed during a period of retarded growth when the foliage buds and catkins become distinguished from each other by slow elongation of the catkins from early summer throughout the fall. Not infrequently the latest formed of these cataphylls become quite leaf-like just as the foliage bud resumes production of foliage leaf primordia in the mid-winter weeks. Buds develop in the axils of foliage leaves and in the axils of many of the transition cataphylls, whereas usually only rudimentary primordia are found in the axils of the cataphylls.

The activity responsible for foliage leaf production merges into shoot elongation and extends over a period from January to early summer. It is particularly interesting that the various stages of shoot and catkin development may not be at all comparable, at any given time, between plants separated only a few miles. Such differences are directly related to peculiar, localized climatic conditions so characteristic of the Pacific coast. Most forms, however, show transition cataphylls at the tips of some of their shoots as the season of active growth ends (Plate II, fig. 3).

The catkins are borne terminally in clusters and range from two to eight or more inches in length when fully expanded. Catkins are frequently found in the axils of both the lower catkin cataphylls and some of the transition cataphylls below a catkin cluster, thus forming a compound ament.

TECHNIQUE.—A total of several hundred buds of *G. elliptica* was collected at weekly intervals for eighteen months. Several collections of *G. buxifolia*, *G. Congdoni*, and *G. Fremontii* were made for comparative studies. The tightly overlapping cataphylls with their dense sclerotic tomentum necessitated

¹ Received for publication June 2, 1942.

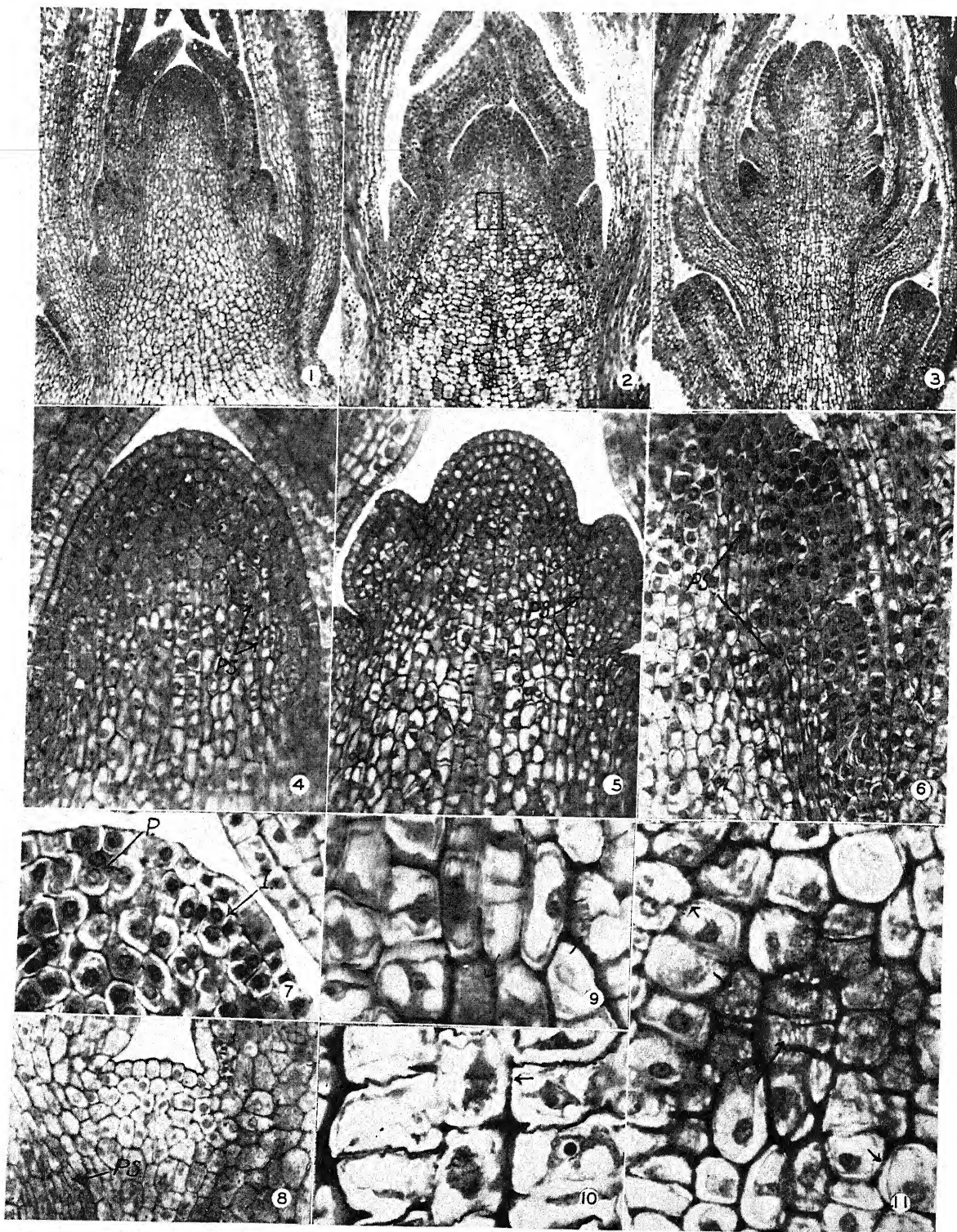


Plate I. Fig. 1-11.—Fig. 1. Longisection of shoot apex during foliage leaf formation (March 10, 1940). $\times 77$. Note the slight elongation of rib-meristem in the region of the oldest node.—Fig. 2. Longisection of shoot apex during cataphyll formation (January 23, 1940). $\times 77$. Note the compacted rib-meristem units and the absence of axillary bud primordia. The rectangle is the area shown in figure 11.—Fig. 3. Longisection of catkin apex showing axillary floret branch primordia of \varnothing florets. Note narrow pith region of axis. $\times 77$.—Fig. 4. Median longisection of the apical dome (of fig. 1) showing early medulation within the apex. $\times 266$. PS—provascular cells.—Fig. 5. Median longisection of shoot apex showing emerged primordia (March 23, 1940). $\times 225$. PS—provascular cells. This apex is cut at a right

careful dissection in order to insure adequate penetration of the killing agents. Formalin acetic acid (or propionic acid) and 70 per cent alcohol in a 5:7:88 ratio and Karpechenko's solution were used as killing agents. All material was aspirated as soon as possible in the reagent for at least an hour. Aspiration was repeated for an hour in the 55°C. paraffin oven after the material had been run up into pure paraffin (cf. Ball, 1941a). The dehydration and paraffin infiltration described by Ball (1941a) was employed.

Prior to cutting, the imbedded material was trimmed to expose a small portion of the base of the bud, and then submerged in distilled water and soaked for two to four days at room temperature, or four to six hours in a 37° oven. This treatment decreased the static generated by the microtome, and also softened the harder tissues sufficiently to diminish tearing appreciably. Longitudinal, transverse, and bias sections parallel to the diverging leaf traces were cut at 4 μ and 6 μ . Tannic acid and iron chloride in combination with safranin produced the most photogenic staining of a number of schedules employed (Foster, 1934).

The cell line drawings were produced by tracing photomicrographs with India ink and then bleaching the photograph in IKI, followed with hypo then water, as described in various photography manuals.

ORGANIZATION AND GROWTH OF THE VEGETATIVE SHOOT APEX.—Apices of fully developed buds vary in diameter at the base of the apical dome from 120 μ to 180 μ . The apices of young lateral buds, in which the early cataphylls are still embryonic, are of smaller diameters. The level of this measurement is not intended to be used as a boundary delimiting the apex. Reference to the apex of *Garrya* may be considered as applying to that distal portion of the shoot on which the youngest pair of primordia has been formed (Plate I, fig. 1, 2, and 3).

In all species examined, the apical dome has a one-layered tunica and an underlying corpus (Plate I, fig. 7 and 8). At certain stages of development it may appear that the tunica is two or even three cell layers deep (Plate I, fig. 4 and 5). Careful examination shows, however, that even during the period of cataphyll formation there are periclinal divisions in the subsurface layer at the summit of the apical dome (Plate I, fig. 7). During the active growing season, the subsurface cells at this region lack consistent uniformity of size and arrangement characteristic of a tunica. Although periclinal divisions are not frequent in this region, they, as well as divisions in variable planes, do occur.

There is a distinct stratified appearance in the corpus following emergence of the most recently initiated primordia (Plate I, fig. 4 and 5). This strati-

fication appears to be correlated with the shifting of activity from the apical dome to the primordia when the apex is in a minimal area stage. Sections cut median to emerging primordia show a decided stratification in the corpus (Plate I, fig. 5). Such stratification is less pronounced in sections median to the next primordia to be produced (Plate I, fig. 4). Before emergence of these primordia, and during the lateral expansion which produces their bases, the cells of the subsurface layer at the summit of the apical dome lack uniformity of size and alignment; both oblique and periclinal divisions may take place.

Variation in tunica stratification has been reported by Boke (1940) for *Acacia*, by Cross (1937, 1938, 1941) for *Fiburnum* and *Vinca*, by Foster (1935a) for *Carya*, and by Schmidt (1924) for *Scrophularia*, and is known to occur in other genera. Cross (1941, 1942) concluded that a variation in the uniseriate tunica of several genera of Gymnosperms constitutes a developmental series which may illustrate the evolution of the tunica as resulting from a reduction of periclinal divisions in the surface layer. Although in these Gymnosperms, the surface layer shows considerable variation, information was not included regarding the relationship of that variability to the periodicity of organ formation, or to seasons of growth.

Foster (1938, 1939, 1941) in his reviews of the problems of shoot growth has pointed out the danger of formalizing Schmidt's (1924) tunica-corpus concept. Although there may be some phylogenetic significance in the occurrence of a multiseriate tunica, the variable stratification in apices on a single plant, as found in *Garrya*, *Acacia*, and other genera, surely indicates a more immediate relationship to shoot growth and organ initiation.

The origin of the tunica in *Garrya* was not determined. Longisections of nearly "mature" embryos constituted the youngest material examined. At this stage the young sporophyte possesses a well-defined surface layer continuous with the uniseriate tunica of the epicotyl apex. The corpus is composed of only a few undifferentiated cells forming a zone about 40 μ to 50 μ in diameter and having the shape of a bi-convex disc (Plate I, fig. 8).

In the summit area of the corpus in a fully-developed apex a central zone of cells may be distinguished as the mother cells of the corpus. Cells flanking and below this zone converge toward it in short, irregular rows of common origin (Plate I, fig. 4 and 5).² The immediate derivatives of the central

² In descriptions of confocal apices most authors speak of cells as "diverging" from a central zone. This viewpoint may have arisen from the classical concept of the apical cell and its "diverging" segments. Since the general direction of shoot differentiation is acropetal, the present writer prefers the term used.

angle plane to that shown in figure 4.—Fig. 6. Longisection of apical flank showing a leaf primordium in an early stage of evagination (March 23, 1940). $\times 290$. PS—provascular cells extending acropetally close to the apical initials of the primordium.—Fig. 7. Median longisection of a portion of the apical dome of a cataphyllary bud. $\times 350$. I—initials of a primordium, P—periclinal division.—Fig. 8. Median longisection of the apex of an embryo. $\times 266$. PS—cotyledonary trace.—Fig. 9 and 10. Oil immersion magnifications of the rib-meristem regions at the levels of younger and older primordia in figure 1 respectively. $\times 500$. Arrows indicate primary pit fields.—Fig. 11. Oil immersion magnification of the medulation zone of the apex in figure 2. $\times 500$. Note the pronounced primary pit fields (arrows).

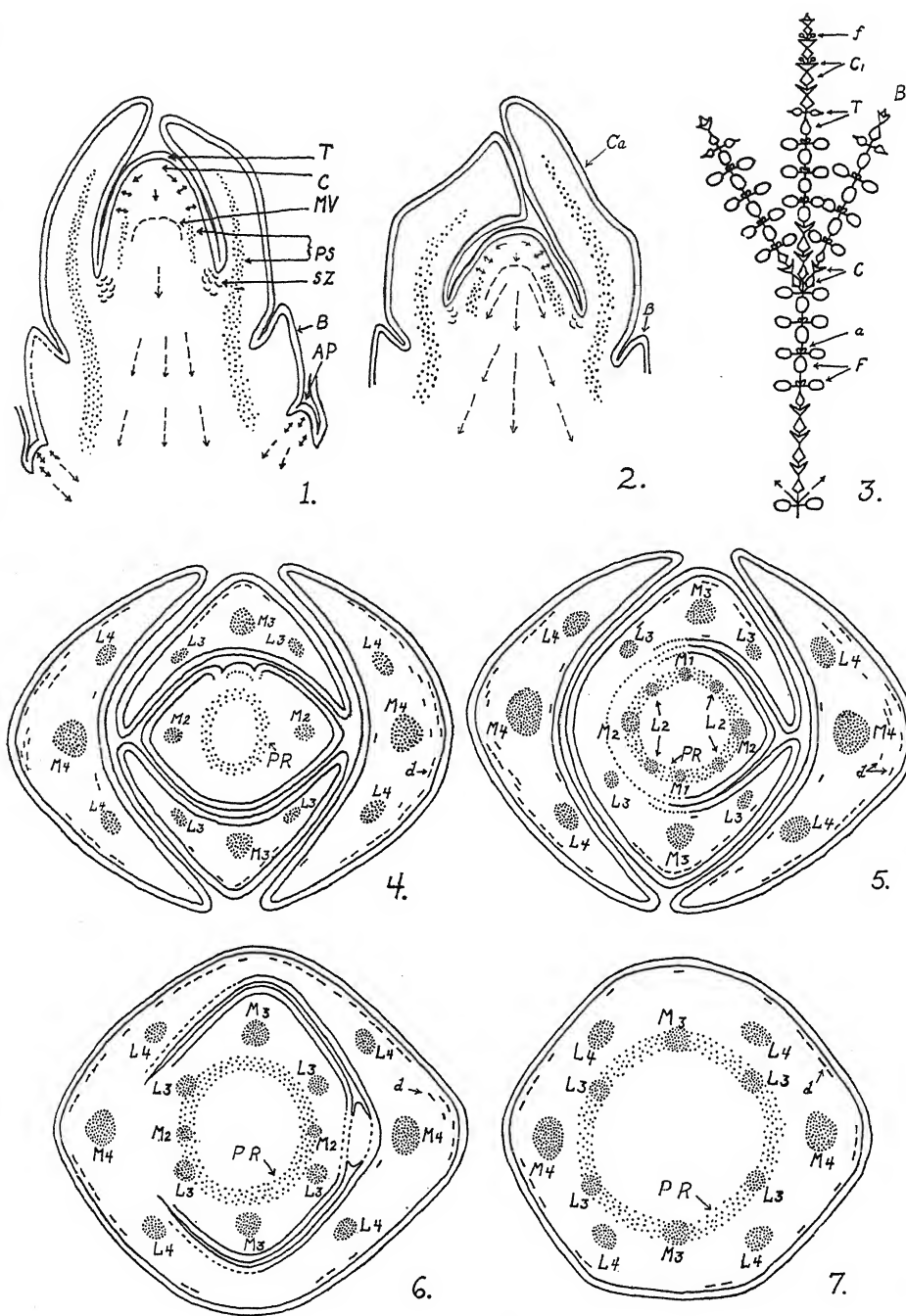


Plate II. Fig. 1-7.—Fig. 1 and 2. Longi-diagrams of shoot apices shown in figures 1 and 2, respectively, in Plate I. *T*—tunica, *C*—corpus, *MV*—approximately the upper limit of medulation, *PS*—provascular strands, *SZ*—shell zone or initials of axillary bud, *B*—base of primordium immediately below and decussate to those shown in the diagrams (this is approximately through the point at which the lateral wings of the primordia unite), *AP*—axillary bud primordium, *Ca*—cataphyll primordium. Small, solid arrows indicate the direction of growth in the corpus. The small, double arrows indicate regions of periclinal divisions which initiate primordia. Broken arrows indicate the cell lineages of rib-meristem.—Fig. 3. Schematic representation of the seasonal production of lateral organs on the shoot. *f*—floret, *C*₁—catkin cataphylls, *T*—transition form, *B*—terminal bud, *C*—cataphyll of vegetative shoot, *a*—axillary bud, *F*—foliage leaf.—Fig. 4-7. Transverse diagrams of figures 3, 4, 5 and 6, respectively, in Plate III. *PR*—provascular ring, *d*—cambial-like divisions of the adaxial and abaxial meristems. The provascular traces are labeled “*M*” for median and “*L*” for lateral, and the numbers correspond to the paired primordia in the order of their ages (thus, primordia I is the youngest).

zone of mother cells are designated in Plate II, figures 1 and 2, by single, solid arrows. This zone forms the precursors of the rib-meristem and the lateral meristem of the corpus from which primordia arise. The more active portion of the corpus is lateral in position (Plate I, fig. 4 and 7). The short, double arrows in figures 1 and 2, Plate II, designate the regions of leaf initiation where periclinal divisions are most abundant. In the immediate vicinity of this region, and differentiating slightly below it, the provascular cells first appear within the apical dome (Plate I, fig. 4, 5, 6; Plate II, fig. 1 and 2). These youngest provascular cells are continuously connected with, and differentiated upward from, older provascular tissue lower in the axis. As such differentiation invades the apical dome it occurs lateral to leaf gap areas of ground meristem, and then the strands of provascular cells converge and "fuse" above the leaf gaps. This provascular tissue may be compared to the tissue described by Louis (1935) as "prodesmogen"; however, its cells are already definitely elongated. The differentiation of this early stage of provascular tissue will be discussed later with reference to the vasculature of primordia.

In Plate II, figures 1 and 2, the dotted line "M-V" presents an approximate division between the more densely staining corpus cells and the region of early medulation or vacuolated rib-meristem (see also Plate I, fig. 4 and 5). The relative extent of these two regions, and the general shape of the apical dome are evidently determined by the amount of mitotic activity and by the particular stage in development of the most recent organs (Plate I, fig. 4 and 5).

Considerable growth takes place in the rib-meristem (Plate I, fig. 9 and 10). At a level two or three nodes below the apical dome highly vacuolated rib-meristem is found; and nearly up to this level, where occasional mitotic figures may still be found, there are large druses occupying nearly the entire volumes of some cells. In the diagrams (Plate II, fig. 2 and 3) the rib-meristem is indicated by the broken arrows. Other characteristics of this tissue are: thickened primary wall areas, primary pit fields, and intra-cellular substance—features similar to those described by Boke (1940, 1941) and Foster (1938, 1939, 1941) for similar meristem.³ These wall characteristics may be observed in the apices of cataphyllary buds at the level of the youngest node (Plate I, fig. 2 and 11), but above that region they are less pronounced. In *Ginkgo*, Foster (1938) has described and illustrated these features within the central mother cell group of the apical dome.

Although the cataphylls and the terminal apex of the catkin are essentially vegetative in nature, catkin ontogeny will be discussed in a later paper. During early stages of development, the organization of the

apical dome of the catkin is the same as that described for the apex of the leafy shoot. The ontogeny of the cataphylls on both catkin and leafy shoot is discussed in the following paragraphs.

ONTOGENY AND VASCULATION OF CATAPHYLL AND FOLIAGE LEAF PRIMORDIA.—These organs are histologically similar during the early stages of their formation, but their initiation and different rates of development are correlated with growth conditions specific to the apices producing them. Retarded shoot growth and retarded activity of the apex are concurrent with cataphyll production. In general, foliage leaf primordia that are still embryonic are longer than cataphyll primordia that have undergone considerable internal differentiation. This difference is evident in primordia between 50 μ and 100 μ long (Plate I, fig. 1, 2, and 3). Provascular differentiation is more rapid in cataphyll primordia than in foliage leaf primordia of corresponding sizes, particularly in later stages of development.

Schüepf (1916) adopted Askenasy's (1880) term "plastochron" to designate the time interval between the formation of two successive sets of primordia in decussate species, or two successive primordia in species with alternate leaves. In this concept the plastochron is associated with the alternation of maximal and minimal areas of the apex (described by Schmidt, 1924). During one plastochron the apex passes from a minimal area into the next maximal area.

In *Garrya* the lateral periclinal lines in the corpus initiating a primordium and marking the end of a plastochron do not add appreciably to the area of the apex (Plate I, fig. 7). If formation of primordia is rapid, then the next pair are appearing as initials in the corpus while the immediately preceding pair evaginate.

According to Louis (1935), the maximal area of the apex is a result of the development of a "sous-bassement" (foliar buttress). In other words, the lateral expansions of the apex result in the formation of new primordia. Further activity within the foliar buttress leads to the upward and outward growth of the primordium.

Two stages of foliage leaf formation are shown in Plate I, figures 5 and 6, and Plate IV, figures 2 and 3. Plate I, figure 7, and Plate IV, figure 1, show the subsurface initials from which a cataphyll will develop. Periclinal divisions beneath the subsurface layer along the upper portion of the buttress initiate the apical meristem of the primordium (Plate I, fig. 5 and 6; Plate IV, fig. 2 and 3).

In stages immediately following the emergence of the primordium, the activity of its apical meristem rapidly extends the primordium into an upper portion histologically distinguishable from the lower portion in longisectional view. The upper third remains more or less undifferentiated (Plate I, fig. 1) in all cells beneath the protoderm until the primordium has reached an age comparable to that of primordia at the fourth node. The lower two-thirds be-

³ The term "meristem" is applied here in the more recent concept rather than in the classical sense by which meristematic cells were defined as being unvacuolated and undifferentiated.

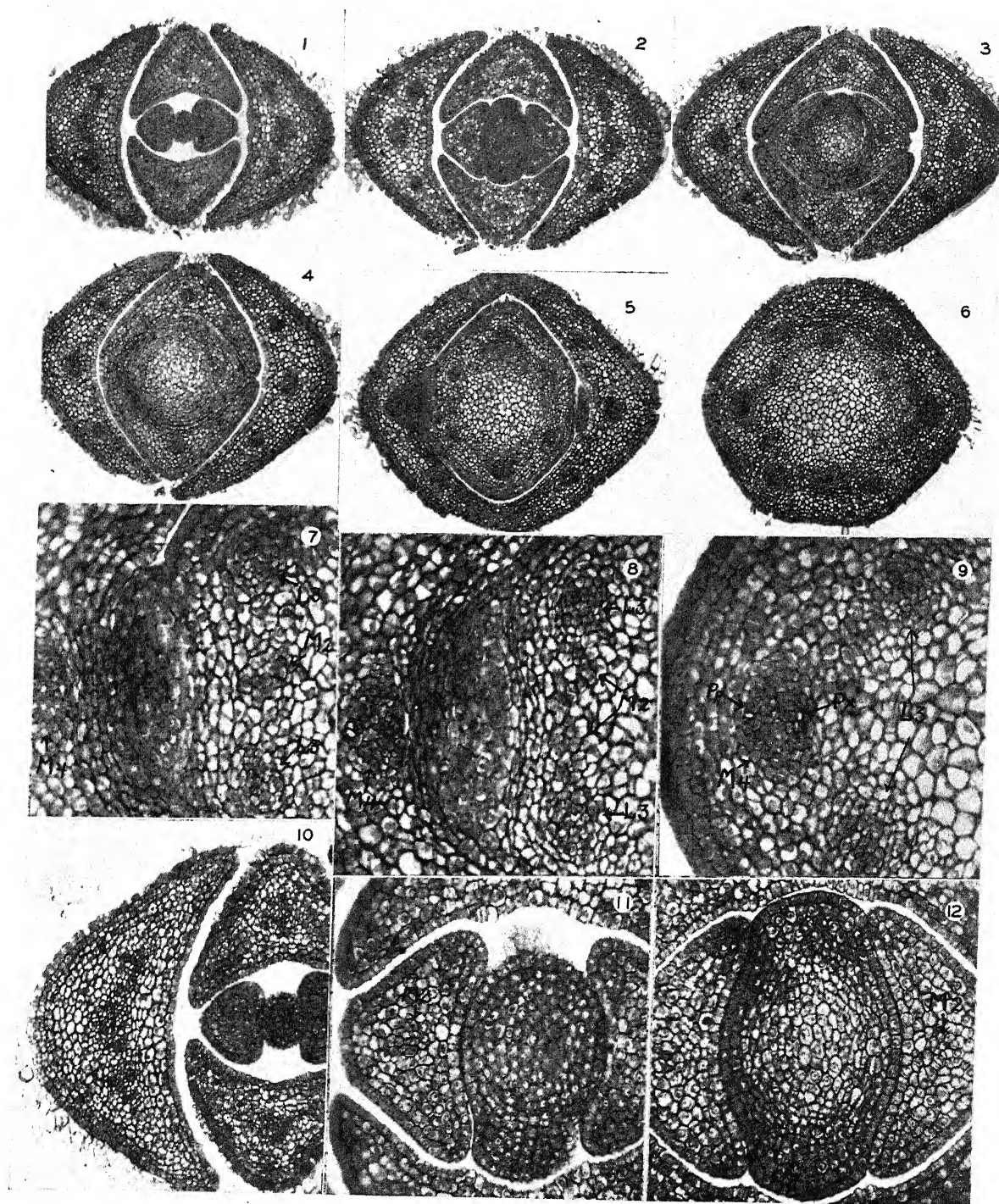


Plate III. Fig. 1-12.—Fig. 1-6. Transverse sections through foliage leaf bud of *Garrya* showing arrangement and association of provascular strands of the primordia.—Fig. 1. Above the level of medulation in the apical dome.—Fig. 2. Through the latest-formed primordia.—Fig. 3. Through the axillary zone of primordia III.—Fig. 4. Through the axillary zone of primordia IV.—Fig. 5. Through the axillary zone of primordia IV.—Fig. 6. Through the bases of primordia IV.—Fig. 7-9. Transverse sections at higher magnification through the axillary level of the primordium shown on the left side of figure 5 and through its base shown in figure 6.—Fig. 7. *L3*—lateral traces to primordia III, *M2*—median trace to a member of primordia IV, *M4*—median trace to a member of primordia IV.—Fig. 8. At a slightly lower level where the branches of the *M2* trace are still distinct.—Fig. 9. Same level as shown in figure 6. *Pp*—protophloem, *Px*—protoxylem.—Fig. 10-12. Higher magnifications of the apical regions of the bud comparable to figures 1 and 2.—Fig. 10. Level

comes progressively more differentiated from the base and insertion of the primordium toward its upper portion. The abaxial and adaxial sides contain vacuolating cells underlying the protoderm and surrounding the vascular strands. In the lower portion of a primordium at this stage of development, Louis (1935) distinguished an abaxial zone of "dorsal parenchyma" and an adaxial zone of "ventral parenchyma," and a central and apically extending "prodesmogen." The "prodesmogen" could well be termed "provascular meristem" in *Garrya* because it is composed of elongated cells and is continuous with the provascular tissue of the axis (Plate I, fig. 5 and 6; Plate IV, fig. 2 and 3). In fact, in *Garrya*, the elongation of the provascular cells occurs even before the abaxial and adaxial zones are clearly defined, and is shown in Plate IV, figure 3, as extending to the apical meristem of the primordium.

Louis (1935) assumed that the procambium differentiated basipetally into the axis and acropetally into the "prodesmogen" of the leaf primordium of *Lonicera*. Grégoire (1938) emphasized that, in contrast to the basipetal differentiation of procambium strands in primordia of foliage shoot apices, the procambium strands of the floral apex and its receptacular tissue differentiate acropetally and without relation to initiation of floral organs. The provascular cells of the foliage shoot apex of *Garrya* do not show a two-way differentiation, since they are continuous with older provascular cells lower in the axis. Moreover, as will be discussed in a later paper, the early differentiation of provascular cells in the axillary floret branch primordia is the same as that found in the early development of the axillary vegetative shoot. In both the differentiation appears to be acropetal.

Boke (1940, 1941) was unable to find any evidence of basipetal differentiation from a "procambial arc" in the phyllode primordia of *Acacia*, and concluded that the provascular differentiation in certain species of cacti is acropetal. Cross (1942) interpreted the differentiation of procambium in *Cunninghamia* as acropetal. To the writer's knowledge, relatively few attempts have been made to study the direction of differentiation of the first-appearing provascular cells in bias longisections cut tangential to the flanks of the apical dome. Schmidt (1924), Louis (1935) and others, however, have recognized that leaf traces are continuous laterally around the "leaf gaps" of the older primordia, but the leaf gap origin as associated with the initiation and earliest vasculature of a primordium has not been demonstrated. In this study, bias longisections proved to be very useful for determining the directional growth of provascular cells. It was also possible to establish that the traces to initiating primordia arise from older provascular cells very near the apical dome.

of figure 1, showing periclinal divisions in the abaxial meristems of the primordia, and cell vacuolation in the third and fourth pair of primordia. $\times 100$.—Fig. 11. About 10μ above the level of figure 2. Note the linear arrangement of cells in the youngest primordium, also the beginning of vacuolation in the adaxial and abaxial regions of primordia II. $\times 225$.—Fig. 12. About 10μ below the level shown in figure 2. Note the early medulation in young axis.

At the beginning of a plastochron certain cells adjacent to the base of the emerging primordium can be distinguished in longisections as the first provascular cells in that region. They continue to elongate as the primordium develops (Plate I, fig. 5 and 6; Plate IV, fig. 2 and 3). As previously mentioned, the first provascular cells within the apical dome may be seen flanking the rib-meristem and extending to the approximate region from which the next pair of primordia will arise (Plate I, fig. 4). Radial and periclinal divisions in this provascular meristem further differentiate it from the ground meristem as the cells of the latter enlarge and become more vacuolate. In serial transverse and longisections, the provascular tissue may be traced through successively older nodes and internodes from its most distal extension downward into the axis without interruption.

This tissue, the future vascular tissue, has been described by Helm (1932) as the "meristem ring." Kaplan (1937) spoke of it, and similarly appearing cells lying between the provascular strands in the axis, as "Rest meristem." The present writer prefers the term "provascular ring," or simply "provascular meristem," because the entire meristem partakes in the formation of the vascular system of the axis. The less differentiated cells within this ring are potential provascular tissue since the interfascicular cambium arises from them. These are less elongated in the early stages of the nodal differentiation and form the parenchyma tissue of the leaf and branch gaps. During initiation of new traces some of the less elongated provascular cells near the apical dome become differentiated as branches from already defined traces of young primordia. These branches differentiate acropetally from their origins and unite to form traces to emerging primordia. Very near the apical dome the provascular cells differentiating into the youngest primordia are at stages of development comparable to the less differentiated cells within the provascular ring of the axis.

Longisections of *Garrya* buds cut tangential to the provascular ring at the base of the apical dome show the cells of well-defined traces as well as younger, less elongated cells (Plate V, fig. 1 and 2). The younger cells may be traced in serial sections to the regions of emerging primordia. Their appearance and differentiation within the provascular meristem proceed acropetally (compare Plate V, fig. 1 and 2, with Plate I, fig. 4, 5, and 6).

In Plate V, figure 2, the provascular cells indicated by the joined arrows lie in the position of a median trace to a member of the next pair of primordia decussate to the one illustrated. Examination of serial sections of this region showed that the provascular cells indicated by the single arrow belong to the edge of the median trace to the primordium illustrated. In other sections additional provascular cells occupy the region of the lateral traces to the

same primordium, and from one of these barely differentiated lateral traces a line of provascular cells diverges toward those indicated by the joined arrows. Thus, before establishment of discrete provascular strands, the origin and location of the future traces of a primordium are already evident within the ring of provascular meristem in and near the apical dome. Further differentiation of these provascular cells and of the traces they form proceeds

acropetally from the ring into the primordia. At the same time, the acropetal differentiation of additional provascular meristem takes place within the ring.

Nodal anatomy.—To illustrate further the relationship of the provascular tissue it is well to consider some of the features of the nodal anatomy of the bud. The organization and the general features of the vascular meristem of cataphyllary buds and of buds producing foliage leaves are similar. The

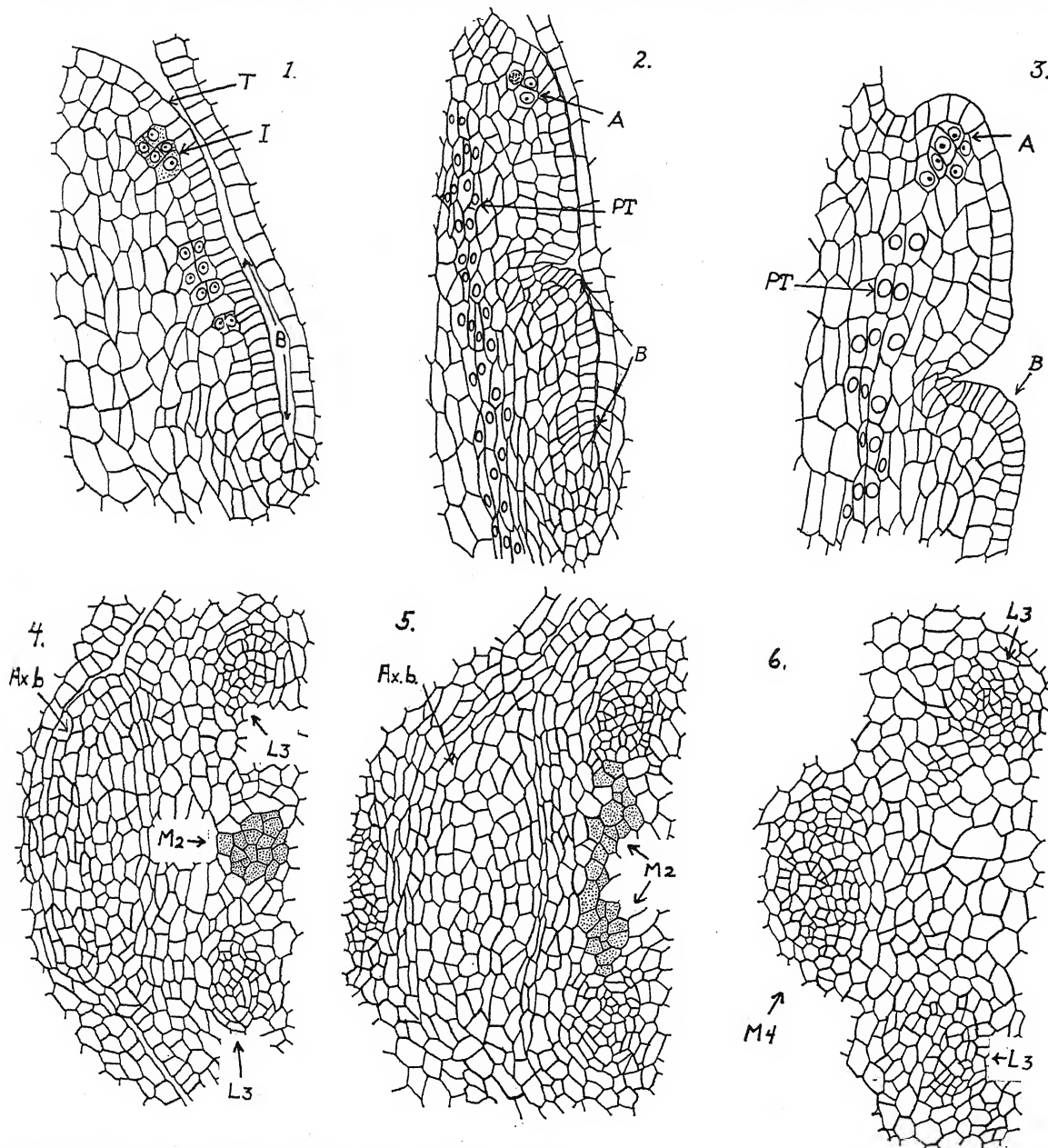


Plate IV. Fig. 1-6.—Fig. 1. Cell tracing of a portion of the apex shown in figure 2, Plate I. *T*—tunica, *I*—initials of a primordium, *B*—basal region of the lateral wings of the most recently formed cataphylls (decussate to "*T*").—Fig. 2. Cell tracing of figure 6, Plate I. *A*—apical initials of foliage leaf primordium, *B*—basal region of lateral wings of second oldest pair of primordia decussate to "*A*," *PT*—provascular trace.—Fig. 3. Cell tracing of figure 5, Plate I (symbols the same as for fig. 2).—Fig. 4-6. Cell tracings of figures 7, 8 and 9, respectively, in Plate III. *Ax.b*—axillary bud (basal region), symbols for median and lateral traces the same as in Plate III.

differences in the rates of tissue differentiation and maturation, as previously mentioned, are related to the seasons of cataphyll and foliage leaf production.

Proceeding acropetally through transverse serial sections from a region five or six nodes below the apex, the course of the provascular traces may be followed from the provascular ring of the axis into the primordia. At the level of insertion of the fourth oldest pair of primordia (*i.e.*, primordia IV), and in the internodal region below them, twelve distinct traces are present (Plate II, fig. 7; Plate III, fig. 6). The inner six of these are the traces of the third oldest pair of primordia (primordia III); the outer six belong to primordia IV. In figure 7, Plate II, the dotted ring "PR" represents the provascular tissue within which the traces to future primordia differentiate. In the diagrams of transverse sections in Plate II, the traces to the younger primordia (M-1, M-2, and L-2) contain cells that are only slightly elongated and are only slightly more differentiated than the provascular cells shown in figures 5 and 6, Plate I. As described by Louis (1935), the cell vacuolation of the central core and of the cortical regions of the axis delimits the ring of "prodesmogen" near the apex. This stage is illustrated in *Garrya* in figure 12, Plate III; however, here the cortical regions show only slight vacuolation and the provascular cells are somewhat elongated (see also Plate I, fig. 4, 5, and 6).

At the level where the inner six provascular traces enter the bases of primordia III, the median traces "M-2" to primordia II are visible and are continuous into the bases of those primordia (Plate III, fig. 11 and 12). Serial sections show that the "M-2" traces arise by a lateral branching from the "L-3" traces lower in the axis (Plate III, fig. 7, 8, and 9). In Plate IV, figures 4 and 5 the stippled cells show the origin of an "M-2" trace. The cell drawing in figure 6 is at a lower level where the branches forming the "M-2" trace have not yet separated from the "L-3" traces.

The traces "M-1" (Plate II, fig. 5) contain provascular cells at approximately the stage of development of those entering an emerging primordium. Likewise, the "L-2" traces are at a comparable stage of differentiation and are scarcely distinguishable from the provascular ring. These groups are not easily followed in transverse serial sections, but they can be determined in bias longi-sections that show the provascular cells of the "M-1" traces separating the "L-2" traces and uniting above a gap composed of ground meristem near the base of the apical dome. This is partially demonstrated in Plate V, figure 2 which shows the provascular meristem on the right side of the section near the location of an "L-2" trace. As previously mentioned, other sections of this same bud show the provascular cells of the "M-1" trace to be connected with the "L-2" traces, and to differentiate acropetally into the base of primordium I on that side of the axis.

Further confirmation of the origins and acropetal extension of provascular traces can be found in the traces of older primordia cut in bias longi-sections.

Figures 3 and 4 of Plate V are sections tangential to a lateral trace of one of primordia III, and show the leaf gap just above a lateral trace to one member of primordia V. Referring again to figure 7, Plate II, the "L-3" traces in the diagram can be followed in serial sections to their origins from the "L-4" and "M-4" traces flanking them. Thus each "M-4" trace contributes to each of the "L-3" traces on its side of the axis.

In Plate V, figures 5, 6, and 7, the bias longi-sections show the origin of an "M-2" trace from the "L-3" traces flanking it. Figures 5 and 6 show the corresponding leaf gap, and also the "M-4" trace at its level of divergence from the axis. It should be noted that the origin of both median and lateral traces to any one pair of primordia occurs at a level about two nodes below their primordia.

Protophloem and protoxylem.—Although a complete analysis of the maturation of protophloem and protoxylem was not attempted, the differentiation and maturation of these elements above the fourth internode will be described.

According to Esau (1938) the protophloem of tobacco differentiates acropetally and continuously from older traces into the young primordia. When the first phloem has reached the level of a primordium base, the first protoxylem appears at the same level opposite the protophloem. Further differentiation of the protoxylem continues both basipetally and acropetally. The order of vascular differentiation in *Garrya* appears to be the same.

Just above the level of insertion of primordia IV (Plate III, fig. 5), the "M-3" traces contain no mature protoxylem and protophloem. At the base of the insertion of primordia IV, each "M-3" trace contains one protophloem element in early maturation. No protoxylem in maturation could be found in the "M-3" traces down to their origin.

On the other hand, the "L-4" traces at a level of the insertion of primordia IV each contain one or two maturing protophloem elements opposite one protoxylem element. Approximately the same degree of maturation was found in these traces up to the upper petiole levels of primordia IV, but in the laminar regions only one or two protophloem elements were found.

The "M-4" traces in the petiole regions of primordia IV each contain three or four protophloem elements. The same numbers of protoxylem elements were found opposite the phloem, but the individual cells appeared to be less uniform in maturation than were the protophloem cells. The maturation of both tissues was approximately the same down into the bases of primordia IV (Plate III, fig. 9).

The main differences in the direction of protophloem and protoxylem differentiation are best illustrated in sections showing their relative times of appearance in the "M-3" and "L-4" traces. Examination of transverse sections of a number of buds showed that the protophloem differentiates acropetally from the axis into the primordia. The protoxylem appearing in either an "M-3" or "L-4" trace in

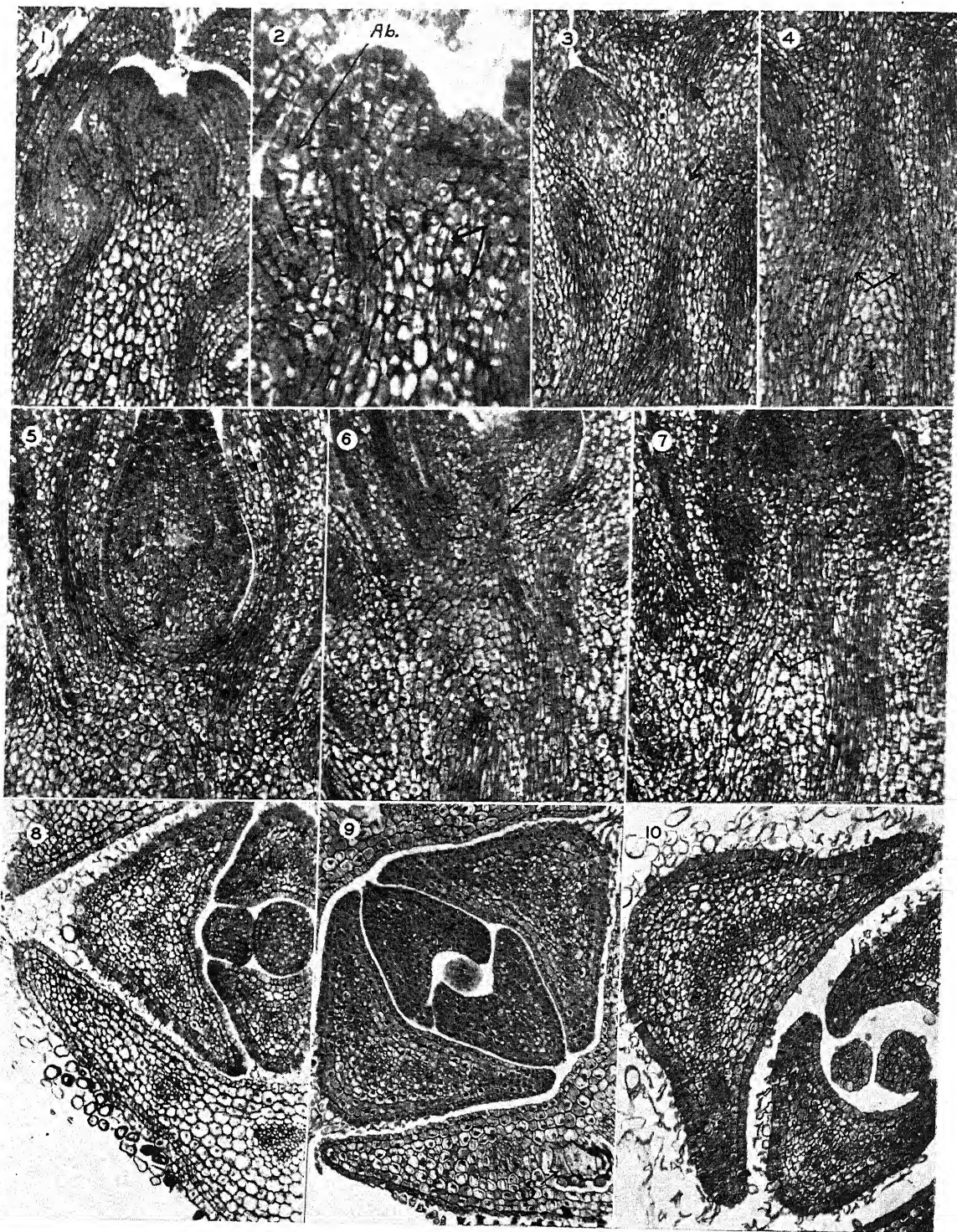


Plate V. Fig. 1-10.—Fig. 1. Bias longisection of foliage leaf bud showing the provascular tissue of a primordium continuous with that of the axis. $\times 120$.—Fig. 2. Higher magnification of the central portion of figure 1. *Ab*—cell vacuolation on the abaxial side of the primordium. Arrows indicate the provascular cells described in the text. $\times 266$.—Fig. 3. Bias longisection tangential to the lateral trace of a primordium $500\ \mu$ long. $\times 120$.—Fig. 4. Bias longisection about $15\ \mu$ inward from figure 4. Arrow below points to a lateral trace to a primordium two nodes below the one belonging to the lateral trace shown above the leaf gap. Joined arrows indicate the branches which fuse to form the lateral trace above the gap.—Fig. 5. Bias longisection tangential to a primordium of the second node ($500\ \mu$ long), arrows indicate a portion of

the internode below its respective primordium is interrupted for a short distance just below the insertion of the primordium. But, at the insertion of the primordium, one or two protoxylem elements are present when the protophloem has reached that level.

The acropetal extension of the protophloem in advance of the protoxylem appears to be correlated with the nature of the first provascular cells. As Esau (1938) has pointed out, there is no sharp distinction between the differentiation of xylem from provascular cells and its early formation from cambium, because provascular cells may divide like cambium. The first protoxylem elements in *Garrya* are differentiated from only a few of the innermost cells of a provascular strand adjacent to the rib-meristem. All subsequent xylem formed prior to obvious secondary growth arises in the innermost region of the provascular groups from tangentially dividing cells. It then appears that most of the provascular meristem which is first evident at the bases of leaf primordia differentiates into phloem. It is even likely that the provascular ring in the base of the apical dome, and the first appearing provascular cells in an emerging primordium are actually "primordial phloem" cells.

INTERNAL DIFFERENTIATION AND MARGINAL GROWTH OF CATAPHYLL AND FOLIAGE LEAF PRIMORDIA.—From the illustrations in Plate III, figures 11 and 12, it is evident that cell vacuolation has already begun in primordia II by the time primordia I are emerging. At the levels illustrated, the abaxial sides of the primordia II are composed of cells already enlarged and somewhat vacuolated. Lateral to the median traces additional cell enlargement and vacuolation have begun, and there are a few subsurface cells on the adaxial sides showing some enlargement and vacuolation. By the time that a leaf primordium has begun apical growth (Plate I, fig. 5), the abaxial side begins to show slight cell enlargement and vacuolation in its ground meristem. About the time the next pair of primordia is being initiated, the ones just preceding are approximately $100\ \mu$ long on the abaxial side and show pronounced cell vacuolation in the abaxial, subsurface region, but this differentiation has scarcely begun on the adaxial side (Plate V, fig. 2 shows vacuolation "ab" on the abaxial side of a primordium in bias section). When primordia are well over $100\ \mu$ in length the abaxial side shows an even greater amount of vacuolation than does the adaxial side (Plate I, fig. 1—note the youngest primordia with distinct traces).

Vacuolation is more rapid in cataphyll primordia than in foliage leaf primordia (compare Plate V, fig. 8 and 10). Cataphyll primordia of catkins show

nearly as much internal differentiation as do foliage leaf primordia nearly twice as large (Plate I, fig. 1 and 3).

While cell vacuolation spreads laterally and acropetally in the primordia, zones of greater meristematic activity are retained in the apical, marginal, and to some extent in the subsurface cells of the abaxial and adaxial regions. Rather than delimit the apical and marginal zones as being composed of subsurface cells only, the apical and marginal meristems are here considered as including the protoderm which is distinct from the subsurface cells only by position and the fact that all cell divisions in it are anticlinal. The apical meristem contributes to growth in length and the marginal meristem contributes to lateral extension of the cataphylls and to laminar extension of the foliage leaves. The abaxial and adaxial meristems contribute to growth in thickness of the basal regions of the primordia.

When cataphylls have reached the stage of development characteristic of the third and fourth nodes, the apical and marginal meristems become progressively less active. Before this stage is reached, lateral evagination from the apical dome is producing the lateral wings of the paired cataphylls. At the same time, the general shape of the young cataphyll has been determined and the lateral wings of the paired primordia become joined; further growth becomes less dependent upon mitotic activity, and more dependent upon cell enlargement and maturation.

The formation of the lateral wings of petiolar bases is quite similar to that just described for cataphylls. The bases of foliage leaf primordia at the second node have united (Plate III, fig. 1-5), but the joined wings are not pronounced until later stages in the development of the primordia. The lateral extension of the petiolar base is never as pronounced as are the wings of cataphylls. Cataphylls of catkins show greater apical extension of lateral wings than do the cataphylls of foliage shoots. These differences are correlated with like differences in the activity of the marginal meristems in cataphyll and petiolar bases.

As appears to be common in Angiosperms, the "pleuroplastic" development (Prantl, 1883) of foliage leaf primordia is found in *Garrya*. Enlargement of the petiolar portion begins when the primordium is about $400\ \mu$ in length, or at the third node, and is preceded by pronounced cell vacuolation in the basal regions of the primordium. A leaf at the fifth node possesses a well-defined petiolar region extending more than a third its length. There is a noticeable reduction in marginal meristematic activity in the pe-

the median trace.—Fig. 6. Bias longisection about $10\ \mu$ inward from figure 5.—Fig. 7. Bias longisection about $20\ \mu$ inward from figure 5. Note the median trace to a primordium two nodes below the one shown in figure 5. Above the leaf gap the arrows indicate the branches of (and their fusion to form) the median trace to the primordium shown in figure 5.—Fig. 8. Transverse section of a cataphyllary bud at the basal level of the apical dome. Note: early vacuolation in the younger primordia, lack of activity in the marginal meristems of older primordia, intercellular thickenings in the mesophyll of the older primordia. $\times 120$.—Fig. 9. Transverse section of a cataphyllary bud showing the tip of the apical dome. S—sclerides. $\times 120$.—Fig. 10. Transverse section through a foliage leaf bud above the apical dome. The oldest primordium shown is cut through the base of the lamina and shows the marginal meristem of the lamina as well as the cambium-like activity in the adaxial, petiole-midrib region. Abaxial activity is less pronounced. Compare with figure 8. $\times 120$.

tiolar region as compared with the lamina. Activity of the marginal meristems in the lamina greatly extends the mesophyll. There is little adaxial and abaxial activity in the laminar region except locally in the midrib area, and even here it becomes diminished. The abaxial and adaxial meristems of the young petiole, however, are quite active.

At about the time the differentiation into petiolar and laminar portions becomes evident, cambium-like activity in the abaxial and adaxial regions of the primordia may be distinguished (Plate II, fig. 4-6, "d"). At this stage of development, these divisions are more pronounced on the abaxial side and extend the full length of the petiole-midrib region (see Plate III, fig. 10). In the lower laminar portion of a leaf at the fifth or sixth node this activity is greater on the adaxial side (Plate V, fig. 10). In a primordium of this age the petiolar region is over 300 μ thick and the laminar region is about 100 μ deep and composed of five to seven mesophyll cell layers. Internal differentiation into palisade and spongy parenchyma does not occur until the primordium is 5 mm. or more in length.

The shifting of activity from the abaxial to the adaxial side of the petiole-midrib in *Garrya* is similar to the type of growth described by Schüepp (1929) for *Acer pseudo-platanus*. It is in contrast to the constant adaxial activity in the young petiole as has been described for a number of Angiosperms by Bouygues (1902), Cross (1937, 1938), Foster (1935 a and b), Prantl (1883), Troll (1935), and others.

The "marginal meristem" described by Louis (1935) as occurring in the axis of the shoot is a continuation of the abaxial meristem of the cataphyll and foliage leaf primordia. In *Garrya*, the abaxial meristem is still active in the lower levels of the bases of older primordia (Plate II, fig. 4-7). It is still present in internodal sections considerably lower than the fourth internode, and is traceable to several inches below the apex where it becomes less evident, but appears to give rise to the phellogen.

In contrast to the foliage leaves, the cataphyll primordia of *Garrya* show considerable activity in the abaxial meristem and only an insignificant activity in the adaxial meristem (Plate V, fig. 8 and 9). This method of growth is quite similar to that described by Foster (1935b) for *Carya* cataphylls. The reduced activity on the adaxial side appears related to the early retardation of meristematic activity in cataphylls and the subsequent more rapid differentiation of internal tissues. Large druses and sclerides appear in the abaxial regions of cataphyll primordia of the fourth and fifth nodes (Plate V, fig. 9).

Some histogenetic features of the marginal meristems.—Both cataphyll and foliage leaf primordia, even at early stages of development, possess a protoderm derived from the tunica, and an inner meristem initiated by the corpus of the shoot apex (Plate I, fig. 5 and 6). Careful examination of the surface layer during all stages of development revealed no periclinal divisions even in the axils; thus the protoderm re-

mains discrete from the time it is differentiated in the embryo. Numerous anticlines in the protoderm increase the surface layer of the primordium.

The apical and marginal meristems of both foliage leaf and cataphyll primordia exhibit only very short rows of cells arising from periclinal divisions (Plate III, fig. 11 and 12), and these are soon obscured by irregular divisions (Plate III, fig. 10; Plate V, fig. 9). This is quite in contrast to the sort of activity described by Foster (1937) for the marginal meristem of *Rhododendron* cataphylls, and by Cross (1936) for *Morus*. Pronounced linear rows of cells arise from periclinal divisions in the margins of *Rhododendron* cataphylls, and in *Morus* short filaments of cells arise from a marginal surface cell. As previously mentioned, the apical and marginal meristems in *Garrya* are regarded as including the protoderm. This general interpretation is supported by the behavior of the protoderm in *Rhododendron* cataphylls because cells are added to the inner tissues by periclinal divisions in the protoderm and the resulting derivatives.

In some features the marginal and apical meristems of cataphylls in *Carya*, described by Foster (1935a and b) are more nearly like those in *Garrya*. In *Carya* the tunica is bi-seriate and the outer layer gives rise to a discrete protoderm while the inner layer contributes to the subsurface cells at the margins of the cataphylls. Short, cambium-like rows of cells are formed by periclinal divisions of the marginal meristem. In *Garrya* the outer cells of the corpus, together with the protoderm, provide the origin of the apical and marginal meristems of the primordia; the cambium-like rows of cells in the marginal meristem, however, are less evident than in *Carya*.

Transition cataphylls.—The transition forms in *Garrya* are similar to those of *Carya* (Foster, 1935b) in periodicity of formation (Plate II, fig. 3). During expansion of the foliage bud, the axillary buds, maturing acropetally, develop several pairs of cataphylls in addition to their prophylls. Transition forms may appear on these axillary shoots just before foliage leaf production begins the next season of active growth. Also, transition forms on the terminal buds may arise as the active season of growth merges into the season of cataphyll production. When a catkin cluster differentiates from a terminal bud, transition forms may be found between the catkin cataphylls and the foliage leaves of the previous year.

Generally those transition cataphylls formed just prior to the season of active growth develop a characteristic aborted lamina that may become as much as two centimeters in length and three millimeters in width. The other transition cataphylls show less development, but they are more elongate and reflexed than normal cataphylls. While no attempt was made to study these transitions in detail, examination of transverse sections of buds collected at the appropriate time of year revealed considerable variation in the amount of activity in the apical and marginal meristems of primordia of comparable ages. Some

cataphyll primordia retain such activity much longer than do others. At the same time, internal differentiation appears to be more pronounced in normal cataphylls than in those produced during the transition period.

The fact that the cell series resulting from marginal meristem activity in *Garrya* cataphylls are less pronounced than those described by Foster (1935a and b) for *Carya* may indicate some relationship to differences in size of the organs in these genera. In normal cataphylls of *Garrya* the activity of the apical and marginal meristems is noticeably less by the time protoxylem can be distinguished in the traces of the primordia, and mitoses are few when the cataphyll is but one millimeter long.

In view of the above observations, the cataphylls of *Garrya* are regarded as equivalent to the bases and lateral sheaths of the petiolar regions of the foliage leaf. This is evidenced not only by histogenetic features, but also by the variability of the aborted laminar portion of the transition cataphyll.

Discussion.—As previously discussed in the paragraphs on the shoot apex, the variable appearance of the tunica-corpus in *Garrya* is correlated with plastochron periodicity, but only one discrete tunica layer is found throughout the annual history of the apex. The fact that variability in the numbers of tunica layers exists in the apices of other Angiosperms serves to emphasize the dynamic aspects of shoot growth. Satina, Blakeslee, and Avery (1940, 1941) working with colchicine-induced periclinal chimeras in *Datura* apices have opened a promising field in which the problems of tissue and organ initiation may be studied more completely.

The tunica of axillary buds forms directly from the protoderm of the axil in *Garrya*. Zimmerman (1928) has shown in *Hypericum* that there are differences in the behavior of tunica layers of axillary buds and terminal buds. In early ontogeny the axillary bud of *H. uralum* possesses three tunica layers; the innermost of these layers, by periclinal divisions, contributes to the corpus and later to the establishment of the third and fourth tunica layers of the shoot apex. On the other hand, the apex of the terminal bud possesses four "permanent" tunica layers. Krumbholz (1925) reported a discrete subepidermal layer in both apex and leaf primordium of *Oenothera*. The subepidermal layer, together with the protoderm, contributes to the apical and marginal meristems of the lateral organs during their early development, but does not lose its identity in the initiation of axillary buds.

Variations in numbers of tunica layers of terminal and axillary buds also have been reported by Schmidt (1924) and other workers. Boke (1940) reported rare periclinal divisions in the "T-2" layer of *Acacia* terminal apices, and regarded variations from a four-layered to a five-layered condition of the apical meristem as "a temporary, divergent development of the outer corpus." That such variations may imply relationships to plastochron periodicity has already been mentioned with reference to *Garrya* apices.

In apices for which a number of tunica layers has been reported, the contributions of tunica and corpus to the lateral primordia remain distinct only until random planes of division in the primordia obliterate these layers. The general mode of initiation of primordia from subsurface layers by periclinal divisions in most Angiosperms differs little from the mode of leaf and cataphyll initiation in *Garrya*. There are, of course, differences in the formation and activity of the apical and marginal meristems of the primordia, but these may be related to size and shape of the mature organs, or to special peculiarities. Monocotyledons also appear to be fundamentally different from Dicotyledons in their apical activity. This difference is evident in such examples as the superficial origin of leaf primordia in *Triticum*, as described by Rösler (1928), and *Avena*, as described by Kliem (1937). In the Palmaceae, however, Ball (1941b) reports that the apices of *Phoenix* are ginkgooid in organization, while *Washingtonia* and *Trachycarpus* possess apices similar to those of the Dicotyledons.

The acropetal differentiation of provascular tissue from the shoot into the primordia of leaves and cataphylls supports the concept of the leaf as a lateral branch system. Arber (1930, 1941) has modified a previous interpretation of plant organs and now considers the leaf and root as partial shoots. As yet, however, an inadequate number of Angiosperms and other vascular plants have been studied to warrant any general conclusions concerning the implications of acropetal provascular differentiation.

SUMMARY

The vegetative apex of *Garrya* possesses a one-layered tunica, discrete throughout its annual history and continuous with the protoderm. Periclinal divisions in the flanks of the corpus initiate decussate primordia. Variations in the stratification of the corpus are related to plastochron periodicity.

The growth of the shoot is much slower during cataphyll formation than during foliage leaf formation. Occasional transition cataphylls occur at the end of each of these two growing seasons. Both cataphyll and foliage leaf primordia undergo apical and marginal growth. Meristematic activity diminishes first in the abaxial and basi-lateral regions and lastly in the adaxial and apical regions of the primordia as progressive cell enlargement and vacuolation take place. Early retardation of the apical and marginal meristematic activity, accompanied by comparatively rapid internal differentiation, distinguishes the cataphyll primordia from the foliage leaf primordia.

The abaxial meristem of the primordia is continuous into the internodes of the shoot. In older internodes, as the bud expands, this meristem appears to form the earliest phellogen.

The petiolar region of the leaf is distinct when the primordium is 500 μ in length and the adaxial meristem has become more pronounced in activity than the abaxial meristem.

The vascular meristem of the cataphyll and foliage leaf primordia is initiated as a ring of provascular cells bordering the rib-meristem of the apical dome. Early differentiation of traces from this ring is associated directly with the initiation of primordia. The median and lateral traces of the primordia diverge from older traces in the axis. These diverging traces differentiate acropetally and join above

the leaf gaps. Their most distal extension in the axis is in the apical dome, quite close to the earliest initiation of primordia, and further differentiation proceeds acropetally into the emerging primordia.

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COMPARATIVE CYTOLOGY OF STERILE INTRA- AND FERTILE INTER-VARIETAL TETRAPLOIDS OF ANTIRRHINUM MAJUS L.¹

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AUTOTETRAPLOIDS, WHETHER of spontaneous origin or induced artificially, are often characterized by reduced fertility. In some cases the most sterile tetraploids are those produced from highly homozygous diploids. Thus in tomato the most sterile tetraploids were obtained from a haploid through the diploid (Lindstrom and Humphrey, 1933) and in maize from inbred lines (Randolph, 1941). A similar condition was reported in *Antirrhinum majus* L. (Emsweller and Ruttle, 1941). Intravarietal tetraploids were relatively sterile and intervarietal tetraploids relatively fertile.

Meiotic irregularity associated with multivalent chromosome association has been cited by Darlington (1937) and Kostoff (1939) as one of the factors responsible for the reduced fertility of autotetraploids. On the other hand Lindstrom and Humphrey (1933) and Randolph (1941) report no correlation between the degree of sterility and the number of meiotic chromosome associations of first metaphase. In view of the above difference in observation the present study was initiated in order to determine, if possible, the relationship of meiotic chromosome behavior to sterility in autotetraploid snapdragons.

CYTOLOGICAL METHODS.—Material was fixed in 2:1 alcohol-acetic acid and placed in cold storage at about 34°F. Two or three changes of the fixative within the first twelve to twenty-four hours seemed to give better results, especially when a large amount of material per vial was involved. Smears were made in iron acetocarmine not less than twenty-four hours after fixation, then heated over a steam bath, pressed and sealed. Longer fixation was preferable, and good results were obtained even after storage for two to four months at 34°F. Sealed smears also kept well in cold storage if placed in a tightly covered container with a layer of 45 per cent acetic acid in the bottom to reduce evaporation. Slides were made permanent by running them up through alcohol-acetic

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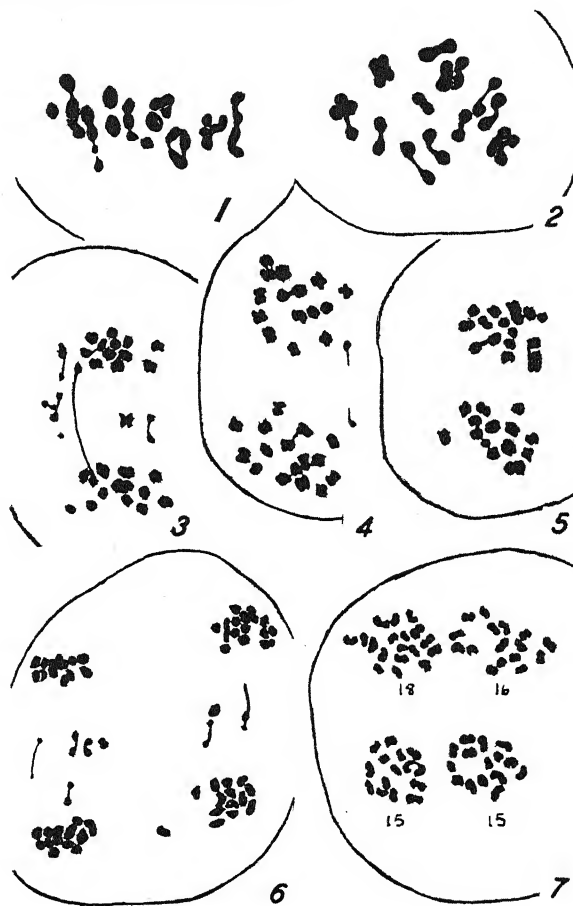


Fig. 1-7. Pollen mother cells of tetraploid snapdragons showing types of irregularities in microsporogenesis which occur in both intra- and intervarietal tetraploids. Camera lucida. $\times 1300$.—Fig. 1. First metaphase showing four quadrivalents, two trivalents (the lower left appearing to have in addition a small fragment), four bivalents and two univalents. Intravarietal tetraploid (40-224-1).—Fig. 2. First metaphase showing four quadrivalents and eight bivalents. Intervarietal tetraploid (41-52-9).—Fig. 3. Irregular first anaphase showing dividing univalents, lagging bivalent and one bridge. Intravarietal tetraploid (41-56-8).—Fig. 4. First anaphase showing fifteen chro-

TABLE 1. *Chromosome associations at metaphase and percentages of unaborting pollen in seven intra- and six intervarietal tetraploids and in one intervarietal triploid.*

Names of original varieties	Plant number	cations per cell Mean number of asso-					Percentage of unaborted pollen
		V	IV	III	II	I	

Intravarietal tetraploids							
Velvet Beauty (V. B.).....	(39-122-1)	0	5.5	.4	4.0	.8	37
Golden Rod (G. R.).....	(40-233-28)	0	5.3	.3	4.5	.9	25
Unidentified (U.)	(41-36-28)	0	4.7	.4	5.3	1.4	67 ^b
After Glow (Af. G.).....	(41-56-8 ^a)	0	5.45	.2	4.5	.6	94
White Wonder (W. W.).....	(41-69-2)	0	4.5	.2	6.0	1.4	60
White Wonder	(41-69-8)	0	4.0	.3	7.3	.5	33
Rose Pink Cheviot (R. P. C.)..	(41-120-4)	0	5.0	.2	5.3	.8	71
Intervarietal tetraploids							
R. P. C. × Pinkie.....	(40-257-1)	0	4.7	.5	5.4	.9	90
W. W. × Alaska.....	(40-281-1)	0	5.2	.3	4.7	.9	95
W. W. × R. P. C.....	(40-285-1)	0	5.5	.1	4.3	1.1	92
V. B. × R. P. C.....	(40-292-1)	0	5.4	.2	4.7	.4	79
V. B. × Red Shades.....	(41-12-71)	.1	5.0	.1	5.5	.7	83
W. W. × U.....	(41-52-9)	0	4.5	.2	6.3	.3	77
Intervarietal triploid							
(Af. G. × R. P. C.) × Torch-light	(41-503-1 ^a)	0	..	6.3	1.7	1.8	45

^a Twenty cells of 41-56-8, and fifteen from 41-503-1 were analyzed for metaphase associations, only ten from other plants.

^b This value is the mean of three sister plants. No pollen was obtained from 41-36-28.

3:1, 9:1, two changes of absolute alcohol and mounting in euparal.

OBSERVATIONS.—The cytological factors which may contribute to the differential fertility in the two types of tetraploids include degree of metaphase pairing, anaphase laggards and unequal segregation at first and second anaphase leading to aneuploid nuclei (fig. 1 to 7). The data in regard to these factors have been tabulated, and differences between the intra- and intervarietal tetraploids tested for significance by the χ^2 or by the standard error method. In some cases coefficients of variability were also calculated and tested for significance.

Chromosome associations at late diakinesis and first metaphase in ten or more cells from each of seven intra- and six intervarietal tetraploids ($4n = 32$) and one intervarietal triploid ($3n = 24$) were analyzed (table 1 and fig. 1 and 2). Table 1 shows that, whereas there is a significant difference in the amount of aborted pollen in the different plants, there is no such obvious difference in the chromosome behavior. Since in most cases only ten cells from each plant were analyzed, the combined data from all the intravarietal tetraploids have been

mosomes in one group, sixteen in the other and, at the right, one dividing univalent. Intravarietal tetraploid (40-224-1).—Fig. 5. First anaphase with fifteen chromosomes in one group and seventeen in the other. In anaphase groups of both figures 4 and 5 there are non-disjoined chromosomes. Intervarietal tetraploid (40-285-1).—Fig. 6. Irregular second anaphase with lagging chromosomes and dividing univalents. Intravarietal tetraploid (41-69-8).—Fig. 7. Second anaphase showing two groups with fifteen chromosomes each, one group with eighteen and one group with sixteen chromosomes. Intravarietal tetraploid (41-56-8).

compared with the grouped data from the intervarietal tetraploids (tables 2 to 4). The frequency distribution of quadrivalents, trivalents, bivalents and univalents in both types is given in table 2, as well as the mean numbers of each with their standard errors.

The mean number of quadrivalents in the intravarietal was 4.99 ± 0.165 and for the intervarietals it was 5.05 ± 0.148 , ranging from 2 to 7² per nucleus. Bivalents ranged in number from none to twelve with means of 5.18 ± 0.296 and 5.15 ± 0.304 in the intra- and intervarietals respectively. Differences in the means in both cases are obviously not significant. The great majority of chromosomes are involved in quadrivalent or bivalent formation (approximately 63 and 32 per cent respectively) while univalents and trivalents together make up only about five per cent of the total (table 3).

Differences in total numbers of quadrivalents, trivalents, bivalents and univalents between intra- and intervarietal tetraploids were also tested by the χ^2 method and found not to be significant (table 3). Nor was there any significant difference between the two types of tetraploids in the percentage of cells which had eight to ten, eleven, twelve or thirteen to sixteen metaphase associations (table 4). (Cells with all chromosomes paired as bivalents would have the maximum of sixteen associations, while cells with all chromosomes in quadrivalents would have only eight.) Variability in number of quadrivalents and bivalents was also tested, but again the differences were not significant.

² Later an occasional cell with eight quadrivalents was observed but none was included in the cells analyzed for metaphase pairing.

TABLE 2. Frequency distribution and means per cell of the main types of meiotic associations in intra- and intervarietal tetraploids.

	Number of cells examined	0	1	2	Number per pollen mother cell										Mean per cell
Quadrivalents															
Intravarietal	80	5	10	13	19	19	14	4.99 ± 0.165
Intervarietal	60	5	16	16	17	6	5.05 ± 0.148
Trivalents															
Intravarietal	80	59	20	1	0.28
Intervarietal	60	48	10	2	0.23
Bivalents															
Intravarietal	80	..	3	13	6	16	4	18	4	9	0	4	1	2	5.18 ± 0.296
Intervarietal	60	2	2	5	2	14	7	14	2	9	0	3	5.15 ± 0.304
Univalents															
Intravarietal	80	42	16	16	3	2	1	0.88 ± 0.127
Intervarietal	60	37	7	13	2	1	0.72 ± 0.132

The number of lagging chromosomes at first anaphase (fig. 3) was scored from at least fifty cells from each plant studied. The percentages of cells with zero, one, two, and three or more laggards per cell are given in table 5. The χ^2 test shows that the number of laggards in the intravarietal anaphases is significantly greater than in the intervarietal ones

somes were frequently noted at late anaphase, especially in cells with unequal segregation. These are, presumably, bivalents or parts of multivalents which failed to disjoin at early anaphase (fig. 4 and 5). Lagging univalents divided at first division (fig. 4) but frequently not in time to be incorporated into the telophase nuclei. Their loss would increase the num-

TABLE 3. Mean number per PMC and percentages of the total number of chromosomes involved in quadrivalents, trivalents, bivalents and univalents in sporocytes from intra- and intervarietal tetraploids.

Tetraploids	IV		III		II		I		Number of cells
	Mean	Percent	Mean	Percent	Mean	Percent	Mean	Percent	
Intravarietal	4.99	62.34	0.28	2.58	5.18	32.34	0.88	2.73	80
Intervarietal	5.05	63.12	0.23	2.19	5.15	32.19	0.72	2.24	60

$$\Sigma\chi^2 = 1.328; \quad df = 3; \quad P \text{ is between .8 and .7}$$

(P is less than 0.01). This is especially true of cells with two or more laggards.

The laggards observed at first anaphase were not all univalents. Bivalents or larger associations were sometimes delayed on the plate and either failed to disjoin or disjoined belatedly. This may partially account for the fact that there is a significant difference in anaphase laggards but not in univalents at metaphase. Associations of two or three chromo-

ber of aneuploid pollen grains and would probably contribute to the higher mean percentage of aborted pollen observed in the intravarietal tetraploids (table 1).

Chromosome counts were made in 268 first anaphases in which all of the chromosomes had moved off the plate and were clearly segregated into two groups. The percentages of cells with equal (16:16) and unequal (17:15 or 18:14) segregations are given in table 6. The mean percentages with uneven segre-

TABLE 4. Percentages of cells with various numbers of metaphase associations (i.e., the total of quadrivalents, trivalents, bivalents and univalents in each PMC) in intra- and intervarietal tetraploids.

Tetraploids	Number of associations				Total number of cells
	8-10	11	12	13-16	
Intravarietal	36.25	22.50	15.00	26.35	80
Intervarietal	35.00	23.33	25.00	16.67	60

$$\Sigma\chi^2 = 3.218; \quad df = 3; \quad P \text{ is between .50 and .30}$$

TABLE 5. Percentages of first anaphases with 0, 1, 2 and 3-5 laggards in the intra- and intervarietal tetraploids listed in table 1.

Tetraploids	Number of lagging chromosomes				Total number of cells
	0	1	2	3-5	
Intravarietal	50.32	25.59	16.20	7.89	469
Intervarietal	62.03	25.67	8.82	3.48	374

$$\Sigma\chi^2 = 20.73; \quad df = 3; \quad P < 0.01$$

TABLE 6. Percentages of equal and unequal first anaphase segregations in intra- and intervarietal tetraploids.

Tetraploids	Anaphase segregation			Total number of cells
	16 + 16	17 + 15	14 + 18	
Intravarietal				
40-233-28	72.0	20.0	8	25
41-36-28	70.0	15.0	15	20
-56-8	79.4	14.7	5.9	34
-69-2	76.0	24.0	0	25
-69-8	72.0	28.0	0	25
Mean	73.9 ± 1.81	20.3	5.8	129
		26.1 ± 1.81		
Intervarietal				
40-257-1	86.7	13.3	0	30
-281-1	80.8	19.2	0	26
-285-1	71.0	29.0	0	31
41-12-71	72.0	28.0	0	25
-52-9	77.8	22.8	0	27
Mean	77.6 ± 2.12	22.4 ± 2.12		139

gations were 26.1 ± 1.81 for intravarietals and 22.4 ± 2.12 for intervarietales. The difference in total number of unequal disjunctions is not significant, but the intravarietals had 5.8 per cent of 18:14 segregations as against none in the intervarietales.

At second anaphase lagging chromosomes frequently occur (fig. 6). Twenty-seven second anaphases without laggards were selected from one intravarietal tetraploid (41-56-8) which had 71 per cent normal segregation at first anaphase. Of these twenty-seven second anaphases seventeen (63 per cent) had the expected number of chromosomes (16:16:16:16), while seven had 17:17:15:15, two had 16:16:17:15, and one (fig. 7) had 16:18:15:15. Of the total of 108 nuclei 35 had either more or fewer than 16 chromosomes, and yet aceto-carmine smears showed that this plant had only six per cent of aborted pollen (unusually low for an intravarietal). In an intervarietales tetraploid (41-52-9) with 70 per cent normal first anaphase segregation, 75 per cent of the 20 second anaphases counted had the expected segregation and 25 per cent had 17:17:15:15. This plant was highly fertile, while the intravarietal one mentioned above, with 32 per cent aneuploid second anaphase nuclei and a lower percentage of aborted pollen, was highly sterile. The extreme differences in seed set are obviously not caused merely by differences in chromosome behavior during microsporogenesis.

The mean percentages of unaborted pollen in the plants examined cytologically and known to have thirty-two chromosomes was 55 ± 9.3 for intravarietals and 86 ± 3.0 for intervarietales (table 1). The corresponding coefficients of variability are 44.6 ± 1.41 and 8.65 ± 2.52 . The difference is significant and indicates that there is a greater variability in the amount of pollen abortion in the intravarietal than in the intervarietales tetraploids. The intravarietals

have a significantly higher percentage of aborted pollen than the intervarietales. The former, however, average more than 50 per cent unaborted pollen, of which a reasonable proportion (up to 70 per cent) will germinate in sugar solution, and yet they set relatively few seeds when selfed. The difference in seed set in the two types of tetraploids is much greater than can be accounted for on the basis of differences in male sterility, i.e., pollen abortion.

Chromatin bridges (fig. 3) were observed in all the tetraploids which were examined in detail. They were usually not accompanied by fragments, although the latter were sometimes present. Second division bridges were also observed in eight of the thirteen plants, despite the fact that comparatively little attention was paid to second division figures. In addition to the bridges which resemble dicentric, some of those seen at first anaphase were obviously the bivalents or quadrivalents, mentioned above, which had stayed on the metaphase plate longer than the rest and were then apparently unable to disjoin normally, and hence remained for a time connected by attenuated strands of chromatin.

Similar bridges are also present in diploid control material grown in the greenhouse. In a clonal line of the variety Alaska the mean frequency of cells with bridges was 0.65 per cent for diploid branches and 1.18 per cent for tetraploid branches. The number of cells examined was 2921 and 1110 respectively.

The cause of the bridges and attenuations is not known, but it may be relevant that Ernst (1938) found numerous translocations, inversions and deficiencies following temperature shock treatment of diploid snapdragons.

It may be added that spindle irregularities not included in the above discussion were observed in three different plants. In one tetraploid and one diploid derived from a colchicine-treated plant, many second divisions had only one spindle instead of two, the chromosomes being arranged in one large metaphase plate, forming two large telophase nuclei in place of the usual quartet. These appeared in the pollen as giant grains or at earlier stages as dyads. In another intravarietal tetraploid many second divisions had more than the normal number of spindles, ranging as high as six. This seemed to be the result of a faulty first division which failed to congregate the chromosomes at telophase. The condition resembles very closely the divergent spindles reported by Clark (1940) in maize, although in her case they arose in the F_2 of a plant fertilized with pollen which had received ultraviolet radiation.

DISCUSSION.—Unequal segregation from quadrivalents has been cited as a cause of sterility in tetraploids by Darlington (1937), Kostoff (1939) and others. Raptopoulos (1941) has reported an inverse relationship between fertility and the mean number of quadrivalents in autotetraploid cherries. Contrary to these observations, the results of Lindstrom on tomato, Randolph on maize and the present studies on snapdragon do not indicate any correlation be-

tween sterility and the number of quadrivalents. Nor can the reduced fertility be attributed to a lack of pollen. Other factors must be operative. Incompatibility and female or zygotic sterility are suggested.

If unequal segregation at first anaphase is due to irregular disjunction from quadrivalents the frequency of non-disjunction can be estimated. The mean number of quadrivalents is five per pollen-mother cell, and roughly one anaphase in four shows unequal segregation. Thus one in twenty of the quadrivalents fails to disjoin normally. The resultant aneuploidy might be considered an important factor in causing sterility in the intravarietal tetraploids were it not for the fact that the intervarietals also have many aneuploid nuclei but are highly fertile.

Unequal segregation from quadrivalents can not be accepted as an explanation for the degree of sterility observed. However, aneuploids occur rather frequently in tetraploid populations of both maize (Randolph, 1941) and snapdragon (Straub, 1941), such plants often being indistinguishable in phenotype from plants with the regular number. Randolph reports that approximately one half the plants in a tetraploid progeny do not have the balanced chromosome number. This is of interest in that our observations show that approximately one in four of the second anaphase nuclei are aneuploid. Assuming that a comparable amount of unequal segregation occurs in macrosporogenesis, then random union of male and female gametes should give nine euploid to seven aneuploid zygotes. The additional aneuploidy resulting from the loss of laggards would bring the ratio up at least to the 1:1 ratio reported for maize.

The data presented above show that the intervarietal tetraploids which give a high seed set also have a higher average percentage of unaborted pollen than the intravarietals. However, even those intravarietal tetraploids, with only a small percentage of aborted pollen, fall far below the level of the intervarietals in fertility. The reduced fertility of the intravarietal tetraploids, therefore, can not be attributed to a deficiency of pollen, although it may be indirectly related.

The differences in the number of 18:14 segregations and in the number of cells with laggards at first anaphase would contribute to the higher percentage of aborted pollen in the intravarietals. But since the degree of pollen abortion apparently is not correlated with seed set, the differences in fertility in the two types of tetraploids can not be explained on the basis of meiotic chromosome behavior at microsporogenesis. It may be assumed, therefore, that differences in their genetic constitution are responsible. An obvious difference is that the intravarietals are *relatively* more homozygous. It seems probable that in tetraploid snapdragons, as in tetraploid maize (Randolph, 1942), homozygosity *per se* may be re-

sponsible for the reduced vigor and fertility of the more homozygous (intravarietal) tetraploids. Conversely heterozygosity would increase the vigor and fertility of the hybrid or intervarietal tetraploids (cf. Huskins and Smith, 1934 p. 393).

SUMMARY

A comparison of microsporogenesis and the percentage of unaborted pollen in intra- and intervarietal snapdragon tetraploids shows that sterility in the intravarietal tetraploids is not correlated with the mean number of chromosome associations, nor with a difference in the mean valency per cell, nor with the valency percentage, nor with group associations; that sterility as measured by seed set is a concomitant of, but is probably not conditioned by, a significantly higher percentage and greater variability in amount of aborted pollen, a significant difference in the number of laggards at first anaphase and a difference in the number of 18:14 segregations at first anaphase.

These minor cytological differences are not sufficient to account for the large difference in fertility of the two types of tetraploids but may stem from the same origin. A relatively higher homozygosity of the intravarietal tetraploids is considered the probable cause of their greater sterility.

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GROWTH HORMONES AS RELATED TO THE SETTING AND DEVELOPMENT OF FRUIT IN *NICOTIANA TABACUM*¹

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STUDIES of growth hormones in plants have revealed many interesting aspects of the problem of fruit development. That growth hormones have a causal relationship to fruit development was first demonstrated when Gustafson (1936) induced parthenocarpic fruit development in tomato, *Petunia*, *Salpiglossus* and pepper. Mature fruits without seeds were produced through the use of the growth promoting chemicals indoleacetic, indolepropionic, indolebutyric and phenylacetic acids applied to the cut style. Gustafson (1937) found that extracts of pollen can induce fruit development in many plants and that this effect is not specific. The results of investigations by Laibach (1932) established the close relationship of the effective substances in pollen extracts to the growth hormones. Extracts of pollen and also of animal tissues caused the gynostemium to swell in certain tropical orchids and promoted the growth in length of the oat coleoptile. These facts were the basis for an hypothesis advanced by Gustafson (1939) that growth hormones from the pollen grains and pollen tubes initiate the early stages of fruit development, and after fertilization the developing embryo provides additional growth hormones. This paper deals with direct measurements of the growth hormones in the style, ovary and pedicel of pollinated flowers.

EXPERIMENTAL METHODS.—The plant used in the experiments has been *Nicotiana tabacum* L., Lizard's Tail variety. It was found that flowers and fruit setting could be obtained continually by cutting back the flowering shoots on plants brought in from the field in October and kept in the greenhouse. The concentration of growth hormones was determined by placing the plant tissue on a block of agar and allowing the diffusion of the growth hormones into the agar to continue for several hours (Went, 1928), and the subsequent application of this block unilaterally to a deseeded *Avena* coleoptile using Skoog's (1937) modification of the standard *Avena* test method (Went and Thimann, 1937).

Pollinations were made at different times previous to the diffusion of the hormones. At the time that some flowers were pollinated, others had the tip of the style covered with small lead foil caps to prevent pollination, and these were used as controls. Only flowers which had the stigma receptive and the anthers in the process of dehiscing were used. The flowers were removed from the plants just before the experiment, and each group of pollinations was

treated as a unit in the diffusion of the hormones. Each pistil was divided into three parts: the ovary, and two equal lengths (1.8 cm.) of the style. The three portions of a single pistil were kept together throughout the experiment so that results for each individual pistil were obtained. Immediately upon excision, the basal cut surfaces of the portions were placed for five to ten minutes on moist filter paper to prevent their drying out until set upon the agar blocks which were 2.7 mm. \times 2.9 mm. \times 1.6 mm. Small supports of copper wire were used to hold the segments of the style in a vertical position, and care was exercised to ensure the existence of a film of water between the cut tissue surface and the surface of the agar. One and one-half per cent agar was used in experiments 1 and 2. The weight of the ovaries forty-five to sixty hours after pollination was great enough to crush the agar blocks to some extent, therefore, 3 per cent agar was used in experiment 3. All diffusions were for a period of three hours in an *Avena* test darkroom at a temperature of 25°C. and in the saturated atmosphere of a large petri dish. At the end of the diffusion period the plant tissues were removed and the agar blocks were applied unilaterally to the deseeded *Avena* coleoptiles. The curvatures of the coleoptiles, after five hours, are recorded in table 1.

Both the styles that were used for the determination of growth hormones and others pollinated at the time of the experiment were dissected under a binocular dissection microscope; the cortex was removed from the style and stigma. The strands of conducting tissue and the pollen tubes were stained with aqueous solutions of magenta red and light green S.F. Yellowish, according to the procedure of Buchholz (1931) and the growth of the pollen tubes was observed.

RESULTS AND DISCUSSION.—*Growth hormones as related to pollination and fertilization.*—Table 1 shows that in only a few instances were appreciable quantities of diffusible growth hormones found in the styles or ovaries of unpollinated flowers. The small amounts and their infrequent occurrence are in striking contrast with the results for pollinated flowers. In the styles from flowers pollinated fourteen hours previous to the diffusion of the growth hormones in the first experiment, the pollen tubes had grown through the apical half of the style and a few millimeters into the basal half. Considerable quantities of growth hormones were obtained from both the apical and basal portions of the styles with those from the basal portions being somewhat larger. The quantities obtained from the ovaries were in most cases less than those from the styles at this time. In the second experiment the growth of the pollen tubes was slower than in the first, the tubes having passed through only the apical half of the style twenty hours after pollination. Smaller amounts of

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TABLE 1. Degrees curvature of individual *Avena* coleoptiles resulting from the application of agar blocks into which growth hormones from portions of styles and ovaries of pollinated and unpollinated flowers had diffused at different time intervals after pollination.

Exp. No.	Hours from pollination to diffusion	Unpollinated flowers			Pollinated flowers		
		Style			Style		
		Apical half	Basal half	Ovary	Apical half	Basal half	Ovary
1.	14	0	0	0	5	5	0
		0	0	0	4	5	5
		0	0	0	5	5	2
		0	0	0	8	9	2
		0	0	5	6	14	5
	42	0	4	0	5	10	14
		0	0	0	1	2	18
		0	0	0	0	4	23
		0	2	0	0	0	30
		4	0	0	0	0	30
		0	5	30
		0	1	33
	11	4	6	0
		3	0	4
		2	1	5
		0	2	5
		7	1	2
2.	20	5	6	4
		2	5	6
		11	10	5
		0	5	7
		0	3	10
	43	0	4	27
		0	6	33
		6	5	0
		10	6	9
		0	..	14
3.	13	0	3	17
		0	2	20
		0	0	16
		0	0	20
		0	0	30
	35	0	0	33
		0	0	26
		0	0	27
		0	3	28
		0	0	30
	66	0	1	5	0	0	26
		0	0	6	0	3	28
		0	0	30
		0	0	30
		0	0	30

growth hormones were obtained from the portions of styles pollinated eleven hours before the diffusion as would be expected. In all experiments the pollen tubes had grown completely through the style and entered the ovary after forty hours when fertilization may be assumed to occur. At this time the apical portions of the styles contained little, if any, growth hormones; the basal portions still had some diffusible hormone while the hormone content of the ovaries had in all except a few instances increased greatly. Poor contacts between the ovaries and agar blocks are probably responsible for the exceptions. It should be noted, however, that two other causes of variation exist in the technique which are of relatively minor importance. The coleoptiles of *Avena* are not all equally sensitive to the growth hormones and

the pollen tube growth of different pollinations is somewhat variable.

That pollination is responsible for the presence of the growth hormones in the style and ovary of the pollinated flower is shown in two ways: first, the hormones are almost entirely absent from the style and ovary of the unpollinated flower; and second, the distribution of the growth hormones is closely related to the extent of the penetration of the pollen tubes into the style. This may be regarded as supporting the hypothesis advanced by Gustafson (1939) which states: "In normal fruits in which pollination and fertilization are necessary . . . the initiation of growth of the ovary into a fruit results from the auxin brought into it by the pollen tubes." However, the critical test of the hypothesis would be

the demonstration of hormone content in pollen grains and pollen tubes comparable to that obtained by diffusion of the pollinated styles. A number of experiments having this as their object were performed with *Nicotiana*, *Datura*, *Antirrhinum* and *Lilium*. Although excellent growth of pollen tubes *in vitro* was obtained, satisfactorily consistent results were not. In no instance was an amount of growth hormones obtained, by the extraction of pollen grains and pollen tubes of *Nicotiana*, which was equivalent to that obtained from the style of a pollinated flower by diffusion.

In evaluating these experiments it must be borne in mind that there is ample evidence to show that the diffusion method and the extraction method do not always give comparable results. It is possible that none of the methods employed here, and which are in general use for the extraction of plant growth hormones, is adequate for comparisons of such a nature.

Van Overbeek, Conklin, and Blakeslee (1941) have recently advanced the hypothesis that, since only a small amount of the total potential growth hormones is present in active form in the coleoptile tip of the seedlings of corn, a similar condition might occur in the young ovary; and "... the diffusible substance from germinating pollen which ultimately causes enlargement of ovaries and ovules may be some prosthetic group, which, when properly combined in the ovary, forms an enzyme which activates the auxin precursor." Skoog and Thimann (1940) have reported what appeared to be the liberation of auxin from proteins of *Lemna* tissue by the addition of proteolytic enzymes. Wildman and Gordon (1942) have shown that auxin is associated with proteins isolated from the leaves of spinach and that the auxin can be released by enzymatic hydrolysis. It is thus possible that the growth hormones found in the style and ovary are not transferred from the pollen but are produced *in situ* as the result of an enzymatic hydrolysis of proteins. To restrict the contribution of the pollen tube to a prosthetic group which combines in the ovary to form an enzyme is unnecessary, for the data presented here show that the pollen tubes do not need to reach the ovary before a considerable amount of growth hormones can be obtained from the style.

Strasburger (1886) found that pollen grains of several plants could hydrolyze a starch paste to sugar, indicating diastatic activity. It was also observed that equal activity was displayed by the intact grains, by grains which discharged their contents, and by germinated pollen. Strasburger's observations were limited to the activity of pollen tubes in the case of *Nicotiana* for the pollen germinated readily in the starch paste medium. Paton (1921) found that in most cases the pollen tubes make their way between the cells of the style by digesting the pectin of the inner lamella with a pectinase. Testing the pollen of eighteen species, she found that all contained amylase, invertase, catalase, reductase and pectinase; pepsin, trypsin, erepsin, and lipase were demonstrated in some and not in others. The inver-

tase and amylase activity of ground pollen was greater than that of unground pollen, while germinated pollen was no more active than ungerminated pollen. The amylase activity of unground germinated pollen was almost as great as that of ground germinated pollen, however. It does not seem improbable, therefore, that along with the other secretions of the pollen tubes during their penetration of the style, there is an enzyme which can release the growth hormones from whatever entities, such as proteins, they may be bound to in the inactive form.

Growth hormones as related to flower abscission.—If pollination and fertilization do not occur, the flowers of *Nicotiana* wither and abscission at the base of the pedicel occurs within three to four days after the flower reaches full development. With such high concentrations of diffusible growth hormones in the fertilized ovary, it seemed probable that appreciable quantities of growth hormones would be continuously moving downward through the pedicel of the fertilized flower. Several experiments were performed to establish this as a fact. The method was essentially the same as that described above, except that only the ovary (without the style) and the pedicel were placed on the agar blocks for diffusion of the growth hormones. The receptacular enlargement of the pedicel was removed and a fresh cut was made at the base of the pedicel before the diffusion.

The curvatures obtained from these diffusions are recorded in table 2. The diffusion in experiments 1 and 2 was into 1.5 per cent agar blocks, while that of experiment 3 was into 3 per cent agar. All were for a period of three hours. The curvatures indicate, as in the preceding experiments, that there is a marked increase in the diffusible growth hormones at the time when the pollen tubes reach the ovary (approximately thirty-five to forty hours after pollination) and at this time, or somewhat later, appreciable, and in some cases considerable, amounts of growth hormones move through the pedicel. Enlargement of the capsule begins about fifty-five hours after pollination under these conditions and larger amounts of growth hormones are present with large quantities moving through the pedicel. The unpollinated controls had no detectable quantities of growth hormones in the pedicels and only small amounts, if any, in the ovaries.

It is concluded from these experiments that the growth hormones from the ovary move through the pedicel and prevent the abscission of the flower by inhibiting the development of the absciss layer, thus allowing the ovary to develop into a fruit. Several investigators working on the abscission of leaves have shown that the growth hormones exert a definite inhibitory effect on the development of the absciss layer. Laibach (1933) found that orchid pollinia inserted in the split ends of petioles of debladed leaves of *Coleus* and several other plants delayed abscission of the petioles twelve to twenty days longer than in the controls. A lanolin paste containing the growth hormones from pollinia of tropical orchids also retarded abscission for eight days. LaRue (1936) ob-

TABLE 2. Degrees curvature of individual *Avena* coleoptiles resulting from the application of agar blocks into which growth hormones from ovaries and pedicels of pollinated and unpollinated flowers had diffused at different time intervals after pollination.

Exp. No.	Hours from pollination to diffusion	Unpollinated flowers		Pollinated flowers		
		Ovary	Pedicel	Ovary	Pedicel	
1.	17	2	0	1	0	
		0	0	5	0	
		0	0	0	0	
	39	4	0	12	8	
		0	0	17	8	
	65	0	1	6	14	
		21	10	
		26	18	
	2.	15	1	0	0	0
0			0	2	0	
0			0	0	0	
36		0	0	8	0	
		2	0	9	1	
		5	0	12	0	
47		0	0	10	0	
		2	0	18	2	
		20	5	
66		23	0	
		2	0	18	11	
		7	0	21	12	
83		29	5	
		22	19	
		32	6	
3.		17	2	0	0	0
			2	0	0	1
			4	0	5	1
	37	0	0	9	0	
		0	0	18	2	
		1	2	18	0	
	60	2	0	18	0	
		0	0	33	9	
		0	0	33	13	
	84	0	0	44	16	
		2	0	17	7	
		23	9	
	28	10		
	35	10		
	38	5		

served that synthetic heteroauxin (indoleacetic acid) applied in agar or lanolin to the cut ends of petioles of *Coleus* and *Ricinus* inhibited the development of the absciss layer for significant periods of time. Similar results were obtained by Myers (1940) who also showed that growth hormones from the leaf blades influence the development of the absciss layer.

The assay of growth hormones by their diffusion into agar emphasizes the mobility they possess. It has been demonstrated that there is a continuous movement of the hormones down the pedicel, while at the same time an approximately uniform concentration of diffusible growth hormones is maintained in the ovary for at least fifty hours after fertilization. That this production and movement of hor-

mones out of the ovary over an extended period of time is important for continued growth of the fruit is indicated by the results of investigations of Gardner and Marth (1937). They found that spraying flowers of *Ilex opaca* with indoleacetic, indolebutyric, indolepropionic, and naphthaleneacetic acids caused fruit set without pollination. However, when a second flush of growth occurred in both pollinated and sprayed plants, the sprayed plants dropped all of their fruit, while none of the pollinated plants dropped their fruit. Gardner and Marth regarded the lack of abscission in the case of the pollinated fruits as probably associated with the development of the embryos within their seeds. That conclusion is substantiated here in so far as fertilization is re-

sponsible for a continuous movement of growth hormones, released in the fruit, through the pedicel.

Another important aspect of the movement of the growth hormones out of the ovary is the part the hormones would have in the development of the conducting elements in the pedicel and other portions of the inflorescence, providing for the movement of food materials into the growing ovary. This development in *Ilex opaca*, according to Gardner and Kraus (1937), is mainly a matter of maturation with only a few additional elements being differentiated from the cambium.

SUMMARY

Direct measurements of diffusible growth hormones were made on styles and ovaries of pollinated and unpollinated flowers of *Nicotiana tabacum*. None or very small amounts were obtained from the portions of an unpollinated pistil, but considerable quantities were found in the portions of a pollinated pistil. The growth hormone concentration was close-

ly related to the extent of the penetration of the pollen tubes into the style—fertilization resulting in the release of large amounts in the ovary.

The data are considered in relation to the hypotheses advanced to explain the initiation of fruit development by pollination. The extraction of pollen grains and pollen tubes did not indicate them to be the source of the growth hormones. It is suggested that the pollen tubes may secrete an enzyme which can liberate the growth hormones from inactive combinations in the style and ovary.

The growth hormones released in the fertilized ovary move downward through the pedicel and prevent abscission of the pistil by inhibiting the development of the absciss layer. These hormones are also probably concerned in the development of the conducting elements through which food materials move into the growing ovary.

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INFLUENCE OF OXYGEN TENSION ON RESPIRATION, FERMENTATION, AND GROWTH IN WHEAT AND RICE ¹

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THE CEREAL grains constitute the most important group of food plants for man. Wheat (*Triticum* spp.) is the leading cereal food of North America and Europe, and rice (*Oryza sativa*) is the chief food of the Orient, where it sustains half the population of the earth. Even in the United States rice has become a crop of considerable importance. Rice is normally cultivated in shallow ponds, filled with water to a depth of six to eight inches during the greater part of the growing season (Jones, 1937). Wheat, however, is always grown under ordinary dry-land conditions, and it is unable to survive more than a brief period of flooding. Submersion in water markedly decreases aeration, and it seems likely that this factor is responsible for the difference in the reactions of wheat and rice to aquatic conditions.

Some fundamental difference between the respiratory systems of these closely related grasses might well be expected to account for their responses to decreased oxygen in the environment. Rice must either be more efficient than wheat in using low concentrations of oxygen, or it must possess a growth mechanism that is only partially dependent upon the limited supply of oxygen. It has long been known that wheat, rice, and other cereal grains can carry on some anaerobic activity.

In an attempt to ascertain the physiological basis for this difference between wheat and rice, a study was made of the influence of oxygen tension, from 0 to 20.8 per cent, on respiration, fermentation, and growth in seedlings of each of these cereal grains.

MATERIALS AND METHODS.—*Respiration studies.*—The studies reported in this paper were carried out during a period of one year, beginning in May, 1941, with seeds of the 1940 crop of Leap's Prolific winter wheat (obtained from A. H. Hoffman, Inc., Landisville, Pa.) and Early Prolific lowland rice (supplied by the Texas Agricultural Experiment Station, Beaumont, Texas). Both are among the leading commercial varieties of these cereal grains. Tests near the close of the work showed that storage of the seeds had produced no significant alteration of the respiratory activity of seedlings grown from them.

Seeds used in the experiments were selected for uniformity in appearance and size. After being soaked for five minutes in a freshly filtered 0.55 molar calcium hypochlorite solution and then rinsed with distilled water, the seeds were placed in large glass culture dishes, on three thicknesses of sterile filter paper moistened with a slight excess of distilled water. These germinators were kept at a temperature of 30°C. in a dark incubator. Approximately three times as many seeds as were required for an experiment were selected and germinated.

Good germination and three-day development of both wheat and rice occurred on filter paper moistened with distilled water. Preliminary tests indicated no important advantage of adding salts or sugar to the germination substrate. Media with ionic balance gave indications of superiority, and the pH value of the substrate had a more marked influence. Wheat germination and development were most rapid on slightly acid substrates (pH 5.0–6.0), while rice development was best in nearly neutral substrates (pH 6.0–7.0).

A period of 25 hours was allowed for wheat germination and a period of 33 hours for rice germination, in order to obtain seedlings of each with roots 5 to 7 mm. long. Secondary roots were evident on the wheat seedlings, but not on those of rice; coleoptiles were developed to a length of 4 or 5 mm.

Groups of wheat and rice seedlings, 25 of wheat or 30 of rice, of the character described were selected and pretreated by soaking them in a sucrose buffer for one hour before they were transferred to each of the respiration vessels charged with the same buffer. The buffer had a pH value of 5.8 and a K/Ca balance of 7.3/1. The buffer solution had the following composition: 0.009 M KH_2PO_4 ; 0.001 M K_2HPO_4 ; 0.0015 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$; 0.117 M sucrose. It was employed in all manometric tests in order to provide a stable medium conducive to maximum seedling activity.

The composition of the buffer was based upon recommendations in the literature supplemented by the tests on seed germination previously mentioned. Numerous studies (Jones and Shive, 1922; Trelease and Trelease, 1935) have shown that good development of wheat occurs at the pH of this buffer. Similar phosphate buffers have been employed by other workers in manometric respiration studies on tissues of higher plants (Marsh and Goddard, 1939; Goodwin and Goddard, 1940; James and James, 1940). Best germination of rice seeds and growth of young seedlings is reported by Alam (1936) to occur at pH 6.4–6.5. Dastur and Winifred (1938) found best growth of rice in the pH range of 6.4 to 7.0. The importance of the physiological balance of salts is well known and has been amply demonstrated for wheat seedlings by Trelease and Trelease (1926). Ion balance has been taken into consideration in the preparation of substrates in a number of recent respiration studies (Goodwin and Goddard, 1940; Turner, 1938a; James and James, 1940). Several investigators have demonstrated increase in respiratory activity, especially anaerobic activity, in substrates enriched with carbohydrates. The maximum effect in barley seedlings resulted when the sucrose concentration of the buffer was 0.117 molar (Brown and

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Morris, 1890; Barnell, 1937; James and James, 1940).

A temperature of 30°C. was adopted for these tests to insure rapid germination and high respiratory activity of both wheat and rice. Tao (1930) described greatest growth of rice at a temperature close to 35°C. Optimum germination temperature for rice was found by Akemine (1914) to be 30–35°C. Dastur and Desai (1935) selected 30°C. for their respiration studies of rice. Mack (1930) showed that wheat produced carbon dioxide most rapidly at 30°C. while developing for 48 hours in a wide range of O₂ tensions. This temperature was somewhat above the optimum, 25°C., for shoot elongation. Shirk and Appleman (1940) chose 28°C. for their studies of the respiration of wheat in a very early developmental stage (28 to 33 hours). The germination of seedlings, the growth studies, the respiration tests and other experiments reported in the present paper were carried out at 30°C., except where specially noted.

After an hour of pretreatment in the sucrose buffer, 25 wheat seedlings or 30 rice seedlings were individually placed on a layer of glass beads, with their roots submerged in the buffer solution to a depth of 1 to 2 mm., in each respiration vessel. Four or five replicates of the same treatment were tested simultaneously. The beads, by holding the seedlings in position, were found to reduce variation among the replicates. Three cc. of the sucrose buffer, added in each vessel, brought the level to the upper surface of the layer of beads.

For measuring the O₂ uptake and the CO₂ production by the same seedling sample, Barcroft-Warburg manometers were used according to the first method of Dickens and Simer (Dixon, 1934, p. 61–67). The sensitivity of the manometer-vessel systems varied, according to the technique used, from 30 to 55 c.mm. for 1.0 cm. of movement of the manom-

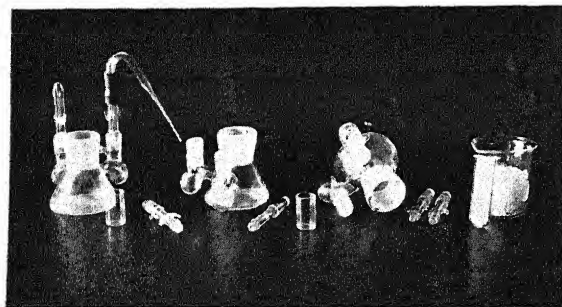


Fig. 1. Respiration vessel. The volume of the closed vessel is approximately 43 cc. The 2.5 cc. side-bulbs, fitted with capillary stoppers, are at an angle of 90° from one another and 45° from the plane in which the vessels are shaken in the bath. Center wells are removable. During flushing with gas, a glass tube is attached to the upper end of the stopper and its open end dips below the surface of the water in the bath. A layer of glass beads covers the bottom of the vessel.

eter fluid. In air (20.8 per cent O₂) 25 intact wheat seedlings consumed about 425 c.mm. of O₂ per hour. The vessels had removable center wells and two side-bulbs fitted with ground-glass capillary plugs (fig. 1). When using gas mixtures with O₂ tensions of 2.5 per cent or higher, CO₂ absorption was accomplished by loading one side-bulb, fitted with a roll of filter-paper, with 0.5 cc. of saturated Ba(OH)₂ and putting 0.5 cc. of 4 N HCl in the other side-bulb. In tests with mixtures containing less than 2.5 per cent O₂ (in which O₂ uptake activity was small in comparison with CO₂ production) both side-bulbs were loaded with Ba(OH)₂ and filter-paper roll, and 0.75 cc. of 4 N HCl was used in the removable center well.

A number of experiments were conducted in which the CO₂ production was determined by means of Warburg's two-vessel direct method (Dixon, 1934, p. 57). The results of such experiments were in good agreement with those in which activity was measured by the technique of Dickens and Simer.

Immediately after the vessels had been loaded with Ba(OH)₂, they were placed in a constant temperature bath maintained at 30±0.02°C. The vessels were connected in parallel to a manifold, and 19 liters of gas mixture saturated with water vapor were flushed through the six-vessel system in 15 minutes. With the aid of a mercury manometer and filter pump, the gas mixtures were prepared by partially replacing the O₂ in normal air with commercial N₂ assayed to be 99.8 per cent. In experiments conducted in complete absence of O₂, the N₂ was prepared by passing the commercial gas through a combustion tube containing 30 cm. of red-hot reduced copper powder.

After being flushed with gas mixture, the closed systems were allowed 10 to 15 minutes to come to the temperature of the bath before the test was started.

During the test the vessels were shaken at a rate of from 100 to 110 complete oscillations a minute through a chord of 3 cm. Uptake of O₂ and evolution of CO₂ were followed, according to the directions of Dixon (1934), over a period of from 45 to 70 minutes, depending upon activity. Immediately after the final readings of O₂ consumption had been taken, the acid was spilled on the substrate and on the tissue, to stop abruptly the respiratory activity. The CO₂ was then released from the BaCO₃ by flushing the alkali bulbs with the acidified substrate. Twenty minutes after the acidification of the Ba(OH)₂, readings were taken of the released CO₂.

After the respiration test, the seedlings were removed from the vessels. Their embryos, including the scutella, were carefully excised from the endosperms and dried to constant weight in an oven at 100°C. Respiratory activities were calculated in the manner described by Dixon (1934). The total embryo weight of the seedlings in each vessel was used in calculating the initial CO₂ production during the preliminary period of equilibration. Original calculation of all activities was carried out on the basis of embryo dry weight.

Since a standard number of wheat or rice seedlings was employed in all respiration tests, it was possible, by means of a conversion factor (total dry weight embryos/number seedlings), to express activities on the basis of units of intact seedlings. Data from 8000 wheat units and 9000 rice units showed that 1.65 milligrams of embryo dry weight were equivalent to one intact wheat seedling, and 0.75 milligram of embryo dry weight was equivalent to one rice seedling.

Growth studies.—Three times the required number of seeds were treated as in the respiration experiments; and after 12 hours, normally developing seedlings were selected for use in experiments on the relation between O_2 tension and growth. Thirty or 35 seedlings were placed on filter-paper moistened with distilled water in a two-quart Mason jar, serving as a growth chamber. The seedlings were allowed to develop in the dark in water-vapor-saturated streams of gas containing from 0.3 to 20.8 per cent O_2 . Six growth chambers, three of wheat and three of rice, were connected in alternate order in a chain. All materials used in the growth chambers were sterilized. Forty liters of commercial N_2 were flushed for 15 minutes through all chains except those supplied 20.8 per cent O_2 ; then all chains, including those with the 20.8 per cent O_2 , were flushed during 20 minutes with 19 liters of the gas to be tested. During the remainder of the growth period, the apparatus was adjusted to a gas flow of 19 liters in 24 hours through each chain.

When the seedlings were 108 hours old, they were removed and fixed in chrom-acetic or 70 per cent alcohol solution. The developed embryos of the group in each chamber were later excised, dried at $100^\circ C.$, and weighed. Increment in dry weight of embryo (i.e., all of the seedling except the endosperm) during the period from 12 to 108 hours was used as a measure of growth.

For germination tests at various O_2 tensions, 100-seed samples of wheat or rice were placed in each chamber of the chain. Germination counts were made at intervals during a period of 72 hours. The results were calculated on a basis of total number of seeds and total number of viable seeds.

In testing the tolerance of wheat and rice seedlings for alcohol, fifteen 12-hour-old seedlings, germinated in the manner described above, were grown in the dark for 96 hours in contact with solutions containing 4 per cent or less, by volume, of ethyl alcohol in distilled water. Six hundred cc., tall-form, spoutless, Pyrex beakers, containing 50 cc. of alcohol solution and lined with two thicknesses of filter paper, served as growth chambers. The seedlings were inserted, between the filter paper and glass, 6.5 cm. above the level of the solutions, and the tops of the beakers were sealed with parafilm. Every 24 hours during a test, the growth chambers were flushed with air, and the alcohol solutions were renewed. All materials used, except the parafilm, were sterilized. Growth measurements were taken as in the experiments on the effect of O_2 tension on the growth of seedlings.

INFLUENCE OF OXYGEN ON GERMINATION.—That crop seeds differ in their capacity to germinate in an environment low in O_2 has been shown in numerous investigations. Crocker (1906) and Shull (1911) reviewed much of the earlier literature on this subject. Morinaga (1926a, 1926b) reported briefly on the germinating capacity of seeds of more than 75 different species of plants and gave detailed results showing that the germination of cattail (*Typha latifolia*) and Bermuda grass (*Cynodon dactylan*) seeds was not inhibited, or was even stimulated, by reducing the O_2 concentration below that in air. He placed wheat seeds in the group incapable of germination under water and cited earlier work showing that rice was able to germinate even in the complete absence of O_2 . Yokoi (1898) and Takahashi (1905) published ample evidence that rice seed is capable of germination under water, in an atmosphere with reduced O_2 concentration, or even in a completely anaerobic environment. More recently, Sasaki (1927) and Edwards (1933) have confirmed the earlier work on rice germination. A decreased O_2 supply—whether existing in the external environment or resulting from the morphological character of the seed—has been considered to have an important influence upon the respiratory activity and germinating capacity of seeds.

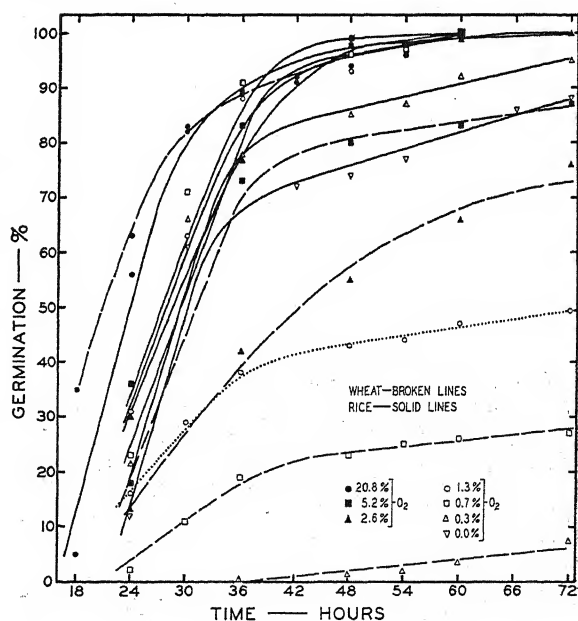


Fig. 2. The germination of wheat and rice seeds in relation to O_2 tension. Wheat, broken lines; rice, solid lines.

In the present investigation it seemed desirable to include experiments showing the effects on germination of progressively reduced O_2 tensions. Data were obtained on the rate of germination as well as on the total germination at the end of 72 hours.

Germination involves the resumption of growth by the seed-embryo. In these experiments seeds were regarded as having germinated when any seedling

TABLE 1. *Percentage germination of viable seeds at various O₂ tensions. Figures represent the average of three tests conducted in each experiment. More than 95 per cent of all readings in all tests deviated less than 10 per cent (the average value in 20.8 per cent O₂ at 7.2 hours=100 per cent) from the average value shown in the table.*

Oxygen tension %	Seed	Average percentage of viable seed germinated in time (hours) noted									
		18	24	30	36	42	48	54	60	66	72
20.8 ^a	Wheat	35	63	82	89	91	94	96	98	99	100
	Rice	5	56	83	89	92	98	98	100	100	100
5.2	Wheat	..	18	..	73	..	80	..	83	..	87
	Rice	..	36	..	83	..	99	..	100	..	100
2.6	Wheat	..	13	..	42	..	55	..	66	..	76
	Rice	..	30	..	77	..	98	..	98	..	100
1.3	Wheat	..	16	29	38	..	43	44	47	..	50
	Rice	..	31	63	88	..	93	97	100	..	100
0.7	Wheat	..	2	11	19	..	23	25	26	..	27
	Rice	..	23	71	91	..	96	97	100	..	100
0.3 ^b	Wheat	..	0	0	0	..	1.5	..	4.5	..	7.5
	Rice	..	21.5	66	78	..	85	87	92	..	95
0.0	Wheat	0	0	0	0	0	0	0	0	0	0
	Rice	0	12	61	..	72	74	77	..	86	88

^a Average of nine tests. ^b Average of six tests.

organ had attained a length of not less than 1.5 mm. By using this criterion, germinated seeds were easily distinguishable by visual inspection of the germinators.

Table 1 gives the results, expressed as percentages of viable seeds, of the germination experiments. The data are presented graphically in figure 2.

It is apparent that reduction of O₂ tension does not significantly reduce germination of rice seeds, whereas progressive reduction of O₂ below 5 per cent produces a rapid decrease in total germination of wheat seeds—no germination at all occurring in the complete absence of O₂. These results are in good agreement with those reported in earlier investigations.

In comparing wheat and rice with respect to rate of germination, we may arbitrarily select a certain percentage of germination—such as 40 per cent—and note in figure 2 the time periods required for this percentage to be reached in the various O₂ tensions by the two types of seeds. It may be seen that the rate of germination of rice is only slightly decreased as the O₂ tension becomes lower. In air 40 per cent of the rice seeds germinated in 23 hours, while in the complete absence of O₂ the time required was increased to only about 30 hours. In wheat 40 per cent germination occurred in air in 18 hours; but a marked decrease in rate of germination resulted when the O₂ tension was decreased. The time needed for 40 per cent germination was 29, 36, and 40 hours, respectively, in 5.2, 2.6, and 1.3 per cent O₂. Wheat seeds were incapable of attaining 40 per cent germination at O₂ tensions below 1 per cent.

INFLUENCE OF OXYGEN ON GROWTH.—In discussing the results of investigations conducted prior to 1900, Pfeffer (1900) showed that growth of aerobic plants is variously affected in different species and organs by decrease in partial pressure of O₂. Since 1900 many conflicting reports on the subject have

appeared. Mack (1930) reviewed much of the important literature dealing with growth and respira-

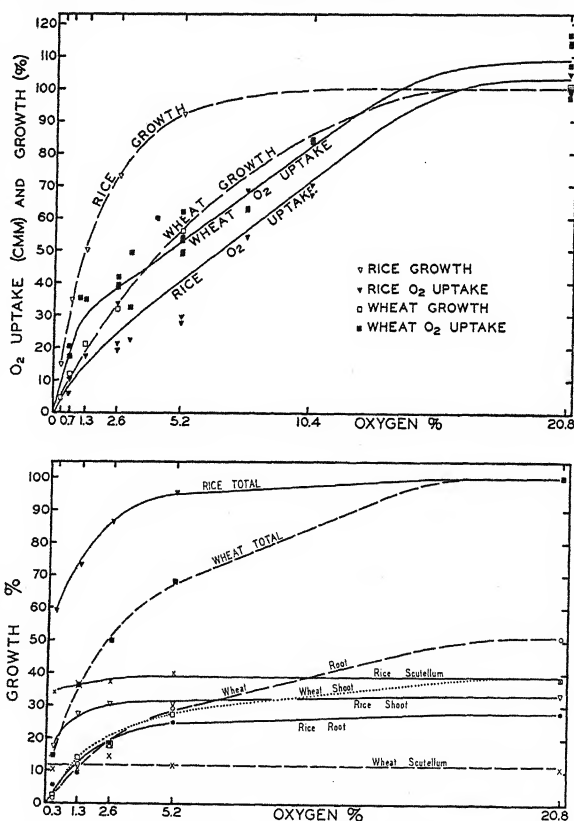


Fig. 3-4.—Fig. 3 (above). Growth and O₂ uptake. The effects of O₂ tension on the increment in dry weight of embryo during the period from the 12th to the 108th hour and on the O₂ uptake of wheat and rice seedlings.—Fig. 4 (below). Growth responses of the parts of intact wheat and rice seedlings to O₂ tension during 108-hour period.

TABLE 2. *Growth of embryo and embryo parts at various O₂ tensions. Temperature of experiments: 1, 30±1°C.; 2, 30±0.5°C.; 3, 29±0.5°C.*

Oxygen tension %	Tissue	Expt. no.	Increment in dry wgt. embryo from 12th to 108th hr.				Average per unit in Expt. No. 3				
			Expt. ave.	Grand mg.	average %	Growth index	Total embryo wgt. 108 hrs. mg.	Fraction of total embryo wgt. 108 hrs. ^a			
								Root %	Shoot %	Scutellum transition zone %	Ratio root/shoot
20.8	Wheat	1	9.30								
		2	10.10	9.20	100	8.3					
		3	8.10				9.00	51.0	38.0	11.0	1.35
	Rice	1	1.75								
		2	1.50	1.30	100	2.6					
		3	0.70				1.10	28.0	33.5	38.5	0.85
5.2	Wheat	1	4.70								
		2	5.10	5.00	56	4.5					
		3	5.30				6.10	29.0	27.5	11.5	1.10
	Rice	1	1.80								
		2	1.10	1.20	92	2.4					
		3	0.65				1.05	25.0	30.5	40.0	0.80
2.6	Wheat	1	2.30								
		2	2.90	2.90	32	2.6					
		3	2.60				4.50	17.5	18.0	14.5	0.75
	Rice	1	1.25								
		2	1.05	0.95	73	1.9					
		3	0.55				0.95	18.5	30.5	37.5	0.60
1.3	Wheat	1	1.60								
		2	1.80	1.90	21	1.7					
		3	2.40				3.30	12.0	14.0	11.0	0.85
	Rice	1	1.00								
		2	0.60	0.65	50	1.3					
		3	0.40				0.80	9.0	27.0	36.5	0.35
0.7	Wheat	1	0.80								
		2	1.30	1.10	12	1.0					
		3
	Rice	1	0.80								
		2	0.55	0.45	35	0.9					
		3
0.3	Wheat	1								
		2	0.20	0.40	4.5	0.35					
		3	0.50				1.40	1.5	2.3	10.5	0.70
	Rice	1								
		2	0.15	0.20	15	0.40					
		3	0.23				0.65	6.0	17.5	34.0	0.30

^a Total weight of 108-hour embryo in air equals 100 percent.

tion of cereal grain crops in early developmental stages. He emphasized the fact that the reaction to a low O₂ supply is dependent upon a complex set of influential variables, including past experience, vigor, current environment, and various counter responses, such as etiolation or respiratory CO₂ output. To obtain evidence on the influence of O₂ on growth, Mack recommended and employed short-interval test periods.

Test periods of short duration were used in the present comparative study of the growth and the respiratory responses of wheat and rice to reduction in O₂ tension. The results of three growth experi-

ments are combined in table 2 and plotted in figures 3 and 4. Three tests, each involving a hundred selected seedlings, were analyzed for each treatment in each experiment. The first two experiments were conducted during the summer, and their results are in good agreement. The third experiment, conducted during the winter, gave similar results, although the absolute growth values were slightly lower, probably because of a somewhat lower temperature. In the first two experiments the increment in dry weight of the embryo from 12 to 108 hours was taken as a measure of growth. In the third experiment the 108-hour weights of the embryo and its parts were used

as the measure of growth; it was not feasible to dissect the 12-hour embryo into its parts.

As the O_2 tension of the environment was progressively decreased, embryo growth of wheat fell off much more rapidly than did that of rice. Considering the increment in both seedlings occurring in air to be normal or 100 per cent, it may be seen that the percentage in rice at any O_2 tension was approximately two or more times as large as in wheat (fig. 3). Rice seedlings were capable of making 15 per cent of normal growth in 0.3 per cent O_2 , while wheat growth was insignificant. It was observed that at this O_2 concentration the length of the shoot of rice was at least 50 per cent of that in air. Macrodissection of shoots of both seedlings grown at the low O_2 tensions revealed that the growth was the result of coleoptile elongation, the plumules having remained undeveloped. Root growth of both seedlings at the lowest O_2 tension tested was almost completely inhibited. The same observation in lesser degree applied to the shoot growth of wheat. In addition, it was apparent that decrease in O_2 tension below 5 per cent had a much more pronounced inhibiting effect upon elongation and lateral development of roots than upon growth of shoots. In view of these observations, it is evident that measurements of growth activity based on shoot length alone, such as were employed by Mack, would not present a complete quantitative picture of the growth reaction, especially in rice seedlings.

A somewhat different measure of the growth resulting in the various treatments may be obtained by considering how many times the weight of the original twelve-hour-old embryos was multiplied during the test period. Growth indices calculated in this manner showed that as the O_2 concentration was decreased to 5 per cent and below, the rate of multiplication of embryo weight decreased at a higher O_2 tension and to a greater extent in wheat embryos than in rice embryos. These two ways of expressing growth—as increment in embryo weight and as multiplication of original weight—yielded results from which similar conclusions may be drawn, in spite of the fact that initially the embryos of rice were only about one-half as heavy as those of wheat.

In the third experiment the growth responses of roots and shoots to decreasing O_2 concentration were noted. The total growth reaction was essentially the same as had been previously observed. In separating the embryo parts, the tissue of the scutellum and transition zone was excised from that of the shoot and root. The results are given in table 3, and they are plotted in figure 4 on a scale of ordinates in which the total weight of the 108-hour embryo in air is considered as 100 per cent. Examination of the curves shows that in wheat seedlings growing in air, root growth is more rapid than shoot growth, whereas in rice seedlings the relationship is reversed. With decrease in O_2 tension, root growth in both seedlings falls off faster than does shoot growth, and the root of wheat is more markedly affected than any other part of either seedling. Of the main growing regions

of the seedlings, rice shoots were least responsive to reduced O_2 tension. There was little or no change in the scutellum-transition zone fraction of either kind of seedling, and so in low O_2 tensions this region constituted the major fraction of the embryo.

In studying the influence of O_2 on the growth of the embryo as a whole as well as on the growth of its components (root, transition zone, and shoot), the use of dry weights resulted in a more accurate picture of the response than would have been obtained by means of a criterion based upon length of embryo or organs, such as was employed by Yokoi (1898) for rice seedlings and by Mack (1930) for wheat seedlings. Although measurements of the linear dimensions of the seedlings or their parts were not made, visual inspection of seedlings at intermediate O_2 tensions showed a shortening and slight thickening of the wheat shoots, accompanied by shortening of primary roots and the development of masses of proliferated root-like tissue where secondary roots would normally have developed. With lowered O_2 tension, where curtailed root growth occurred in rice, the linear growth and the lateral branching were decreased. In other respects these rice roots appeared nearly normal, and they were similar to those diagrammed by Yokoi. While retarded elongation of

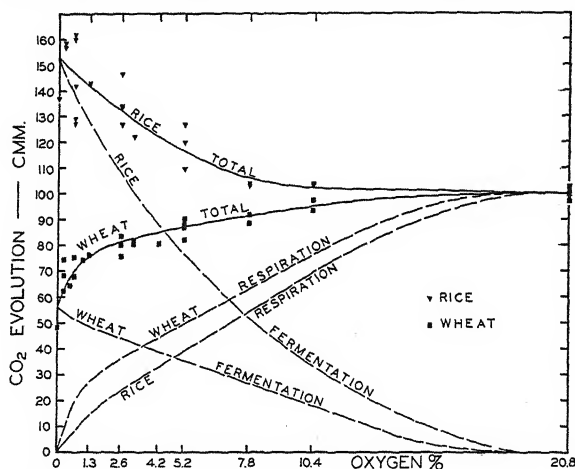


Fig. 5. The influence of O_2 tension on the evolution of CO_2 by intact seedlings of wheat and rice. Total CO_2 , respiration CO_2 , and fermentation CO_2 are shown.

rice roots was occurring in response to O_2 reduction, the shoot elongated at an approximately normal rate.

The results of the present study on total embryo development of wheat confirm and amplify the observations reported by Mack (1930). The growth reactions of rice here recorded are in good agreement with those described in less comprehensive studies by a number of investigators, including Yokoi (1898), Nagai (1916), Sadamoto (1926), Sasaki (1927), and Edwards (1933).

RESPIRATION OF INTACT SEEDLINGS.—Most seeds normally germinate and accomplish early development in environments known to contain low O_2 tensions, intermediate between that of normal air and

the completely anaerobic condition. Under reduced O_2 tensions, various types of seeds might be expected to show differences in their aerobic-anaerobic metabolic balance. It is evident that rice seeds are capable of germinating in the absence of O_2 and the seedlings grow well in an atmosphere containing a very low O_2 tension, whereas wheat is unable to germinate or develop under similar conditions. In attempting to explain the marked differences in the ability of various seeds to grow in low O_2 concentrations and the differences in the germination and growth of the wheat and rice seedlings observed in the present studies, it seemed desirable to investigate the influence of variation of O_2 tension on the respiratory activities of these seedlings.

The literature contains many reports of effects of O_2 on the growth or respiratory activity of seedlings of higher plants. Among the earlier papers are those of Pfeffer (1885), Diakonow (1886), and Palladine (1894). These investigators noted that in a very few cases the anaerobic CO_2 output of germinating starchy seeds equalled or even exceeded the aerobic CO_2 output. The early work, largely by German and Russian investigators, on the respiration of young seedlings has been considered in detail in treatises by Palladin (1926) and Kostychev (1927). These studies showed that seedlings of higher plants are capable of maintaining various degrees of CO_2 evolution over short periods of time when they are subjected to low O_2 tensions or even to completely anaerobic conditions. Wheat was placed in the group having an average or low anaerobic activity. In a detailed study of the higher plants, Stich (1891) reported that rice seedlings were capable of high CO_2 outputs at O_2 tensions below that in air. Mack (1930) carried out a comprehensive investigation of CO_2 evolution and simultaneous growth response of wheat seedlings. Dastur and Desai (1935) studied changes in respiratory activity and carbohydrate reserves in rice seedlings from germination to the ten-day stage. James and James (1940) made a thorough study of the course of respiration and metabolic change occurring in barley seedlings. In the three studies just mentioned, macro-methods were used to follow the respiratory activities of seedlings exposed continuously, from the beginning of germination, to controlled gas environments. The more sensitive manometric technique was used by Shirk and Appleman (1940) and Merry and Goddard (1941), to follow the O_2 uptake or CO_2 output of seedlings of wheat or barley. A number of studies on aerobic respiration have been reported in which either the evolution of CO_2 or the uptake of O_2 was observed, and some papers have dealt with the anaerobic CO_2 output in the absence of O_2 . A complete picture, however, of changes in the respiratory complex— aerobic and anaerobic activities—that occur in response to decreasing O_2 tension cannot be obtained from measurements of CO_2 output alone.

In the present investigation manometric measurements, during short intervals of time, were made of the effects of O_2 tension on the respiratory activities

of intact wheat and rice seedlings germinated in air and then treated with the experimental gaseous environment immediately prior to and during the actual period of the tests. Measurements of both O_2 uptake and CO_2 output, during the same period, were made according to the method of Dickens and Simer.

The results of the gas-exchange measurements are shown in table 3 and figures 3 and 5, in which smoothed curves have been drawn in solid lines to show the trend of the experimentally determined points, each of which represents the average of four or five separate determinations. In 20.8 per cent O_2 (air) the average activity of rice seedlings, as shown by CO_2 evolution, was found to be 87 per cent of the average of wheat seedlings on the basis of dry weight of embryos. To facilitate comparison of the experimental data, the curves in all cases have been plotted for the activity of a number of seedlings (wheat, 6.4; rice, 13.3) calculated to produce 100 c.mm. of CO_2 per hour in 20.8 per cent O_2 .²

Inspection of the curves of figure 3 for O_2 consumption reveals the following relations: The O_2 intake by rice is less rapid than that by wheat over the range of O_2 tensions in which tests were conducted. With decrease in O_2 tension, the O_2 uptake curve for wheat falls rapidly and roughly parallels the curve for growth. In rice the relation is very different: As the O_2 tension is decreased, the growth curve remains relatively high in spite of a rapid and continuous decrease in the O_2 intake.³

In spite of lower aerobic respiratory activity, rice seedlings were able to make more nearly normal amounts of growth than wheat seedlings in O_2 tensions lower than that of air or in the complete absence of O_2 . To account for the difference in growth response, we might assume greater utilization by rice of the energy liberated in respiration; but such an assumption is unsupported by experimental evidence. It seems more logical to seek an explanation of the difference in growth response in a difference in the fermentation (anaerobic) activities of the two types of seedlings.

Figure 5 shows not only total evolution of CO_2 , but also its components—respiration CO_2 and fer-

² The values for O_2 uptake activity of wheat seedlings in air are slightly lower than those obtained by Curry, Pratt, and Trelease (1935) for similar material; but, probably owing to differences in stage of seedling development, they are five to seven times as great as those reported by Brown and Goddard (1941) and by Shirk and Appleman (1940).

³ During the course of these experiments, a large number of tests were made with NaN_3 (sodium azide) in the substrate. Approximately 70 per cent of the normal activity in wheat and 50 per cent of the normal activity in rice was inhibited by 10^{-4} M NaN_3 . Significant photo-reversible inhibition of the activity of both seedlings was observed at 20°C. in a 90 CO_2 /10 O_2 gas mixture compared with a 90 N_2 /10 O_2 mixture. These results indicate that respiration in these seedlings is at least partially dependent upon cytochrome oxidase. From similar evidence it has been concluded that this enzyme is probably responsible for a large fraction of the respiration of barley seedlings (Merry and Goddard, 1941) and of wheat embryos (Brown and Goddard, 1941).

TABLE 3. *Respiratory activities of intact wheat and rice seedlings at various O₂ tensions. (C.mm./hour/standard sample: Wheat 6.4 seedlings, rice 13.3 seedlings.) R.Q. values in air: Wheat 0.92, Rice 0.96.*

Oxygen tension %	Experimentally determined				Calculated from values on smoothed curves for experimentally determined activities							
	Total O ₂ uptake		Total CO ₂ evolution		Respiration CO ₂ evol.		Fermentation CO ₂ evol.		Meyerhof quotient		Ratio F/R	
	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice
50.0 ^a	106.0	146.0	81.0	125.0								
20.8	113.6	113.7	97.0	102.0	100.0	100.0	0	0	0.6	1.5
	116.3	104.4	100.8	98.2								
	107.2	99.4	101.7	99.6								
	97.6	98.2	99.4	102.2								
10.4	84.6	67.0	97.2	103.4	76.0	69.2	19.2	32.9	0.5	1.7	0.3	0.5
	83.8	70.0	93.2	102.6								
7.8	63.0	54.0	91.6	103.4	62.1	54.1	28.7	52.6	0.4	1.9	0.5	1.0
	62.6	68.6	88.4	102.6								
5.2	61.9	29.5	88.0	109.1	48.8	39.1	38.5	78.1	0.4	1.9	0.8	2.0
	49.3	27.7	86.8	119.6								
	54.0	49.5	90.0	126.4								
	53.1	82.0								
4.2	60.0	80.7	43.6	32.3	40.6	89.5	0.4	2.0	0.9	2.8
3.1	49.4	22.0	80.4	121.8	38.0	26.7	44.2	102.6	0.3	1.9	1.2	3.8
	32.5	82.0								
2.6	41.8	33.4	83.5	146.0	37.7	23.5	46.0	108.7	0.3	1.9	1.2	4.6
	39.1	21.1	75.8	133.0								
	38.4	19.0	80.0	126.5								
	39.5	133.3								
1.3	34.9	17.5	76.4	143.0	27.8	13.4	49.0	128.5	0.3	1.8	1.8	9.6
1.0	35.3	74.1	25.0	11.2	50.5	132.5	0.2	1.8	2.0	11.8
0.7	20.4	10.3	68.0	126.6	18.6	8.4	52.0	138.5	0.2	1.7	2.8	16.5
	17.3	5.8	75.4	159.5								
	128.4								
	10.5	141.3								
	9.7	162.0								
0.5	64.6	12.9	5.6	52.5	143.0	0.2	1.8	4.1	25.5
0.3	74.4	158.5	6.0	2.8	53.5	147.5	0.4	2.0	8.9	52.7
	62.4	156.7								
	158.0								
	0.3 ^b								
0.0	0	0	48.4 ^c	136.3 ^c	0	0	56.0	153.0	0	0

^a Average of two tests. ^b Average of five tests. ^c Average of six tests.

mentation CO₂. In preparing these curves, two basic assumptions were made: The first assumption was that in 20.8 per cent O₂ (air) all the CO₂ production of the seedlings was due to respiration (aerobic activity)—in other words, that in this O₂ tension no fermentation (anaerobic activity) occurred. The second assumption was that in all O₂ tensions the aerobic respiratory quotient (R.Q. = CO₂/O₂) was the same as it was in air.

The assumption that in 20.8 per cent O₂ all the activity is due to respiration (and only a negligible amount due to fermentation) is supported by many investigations on normal tissues of higher plants (Blackman, 1928; Kostychev, 1931; Turner, 1937; Marsh and Goddard, 1939; Merry and Goddard, 1941). It is well known that wheat and rice seedlings

contain starchy reserves which are converted to sugars and are then aerobically oxidized according to the general equation: $C_6H_{12}O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 H_2O$. This results in a theoretical R.Q. (CO₂/O₂) of 1, which has been approximated in many studies (Tao, 1930; Dastur and Desai, 1935; Rianga, 1938; James and Bunting, 1941). In air the R.Q.'s of wheat and rice seedlings, both intact and excised, in the large number of tests and control determinations conducted in the present study did not vary markedly from 1, and so indicate aerobic respiration of hexose sugar. In tests made in 50 per cent O₂ the R.Q. for wheat seedlings was found to be 0.80, and for rice seedlings 0.85, whereas in air the R.Q.'s were 0.92 and 0.96, respectively. The lowering in the R.Q. resulted from a slight decrease in

CO₂ output and a slight increase in O₂ uptake. These results indicate that a small but probably negligible amount of fermentation may have occurred in air, possibly because of a slight limitation of O₂ diffusion. R.Q. values of 1 might result from combinations of various reactions involving aerobic and anaerobic respiration of heterogeneous substrates containing sugars, proteins, or fats, and non-enzymatic, non-respiratory oxidations (Goodwin and Goddard, 1940). However, under the conditions of the present experiments and in view of the findings of other workers, it seems reasonable to assume that the observed R.Q. values of approximately 1 for seedlings of wheat and rice in air represent chiefly aerobic hexose catabolism.

The second basic assumption made in preparing the curves of figure 5—namely, an essentially constant R.Q. for respiration (aerobic activity) at all O₂ tensions—seems entirely logical in the absence of any evidence indicating that a different type of substrate is consumed in respiration at lower O₂ tensions. The assumption of a constant R.Q. for aerobic respiration has been made in many similar investigations.

Continuing with the description of the method of calculating the data presented in figure 5, it is evident that knowing the respiratory quotient, the measured O₂ absorption, and the CO₂ evolution over the range of O₂ tensions, it is possible to calculate the amounts of respiration CO₂ and fermentation CO₂ for any O₂ tension as follows:

C.mm. O₂ × respiratory quotient = c.mm. respiration CO₂.

C.mm. total CO₂ — c.mm. respiration CO₂ = c.mm. fermentation CO₂.⁴

Applying this method to the smoothed curves for total CO₂ (fig. 5) and O₂ (fig. 4), the curves for fermentation CO₂ shown by broken lines in figure 5 were obtained.

From figure 5 it may be seen that as the O₂ tension decreases, the experimentally determined curve for total CO₂ output of wheat falls, whereas there is a rise in the corresponding curve for rice.

The greater CO₂ evolution in the absence of O₂ than in air is comparable to what was observed in *Vicia faba* in earlier studies (Diakonow, 1886) and is more pronounced than the reaction noted in rice by Stich (1891). Morinaga (1925) and Dastur and Desai (1935) found high anaerobic CO₂ output in rice seedlings subjected to relatively long anaerobic pretreatment, but this did not exceed the aerobic CO₂ output. Differences in experimental technique

⁴ Difficulties would probably be encountered if attempts were made to differentiate quantitatively between respiration and fermentation in complex heterogeneous seedling tissues by use of respiratory block techniques. The results might be complicated by general toxic effects of the respiratory inhibitors on the protoplasm, and they might depend upon the character of the heterogeneous tissues studied and upon the point at which the common pathways of respiration and fermentation processes branch. Some aspects of this problem have been treated by Turner (1937, 1938b), Marsh and Goddard (see Burk, 1939, p. 457), and James and Bunting (1941).

—i.e., in substrate, pretreatment, material, duration of the test, or method of analysis of gas exchange—may well account for the differences in activities observed in various studies. The rate of aerobic CO₂ output of wheat seedlings in air noted in the present experiments was identical with that observed by Mack (1930) for seedlings of the same age and at the same temperature.

The curves for CO₂ produced in respiration are similar in form to the O₂ activity curves of figure 3 from which they were calculated; wheat shows higher respiratory activity than rice.

Inspection of the fermentation curves of figure 5 shows about 2.7 times as great activity in rice as in wheat. As the O₂ tension decreases from 10 to 0 per cent, the fermentation in rice increases rapidly, until the CO₂ output by fermentation in 0 per cent O₂ reaches 150 per cent of the total CO₂ evolution in air. With corresponding decrease in O₂ tension, the increase in fermentation in wheat is much more gradual, and in the complete absence of O₂ the CO₂ output attains a level only 57 per cent as high as the total CO₂ output in air. This observation is in complete agreement with that of Karlsen (1925).

Approximate anaerobic CO₂/aerobic CO₂ ratios (total CO₂ in N₂/total CO₂ in air) for wheat and rice are 0.5 and 1.5, respectively. For wheat this value is the same as values found by many earlier investigators. The value of the ratio in rice is extremely high and far above those reported for most other seedlings or tissues of higher plants. In *Vicia* seedlings Diakonow (1886) found a ratio of 1.5, and others have obtained values near unity for *Vicia* and *Pisum*. More recently, Marsh and Goddard (1939) found that carrot roots had a ratio of 1.2.

Anaerobic CO₂/aerobic CO₂ ratios and Meyerhof quotients greater than 1/3 have been interpreted as indicating the operation of the Pasteur effect—that is, O₂ inhibition of fermentative processes (Dixon, 1937; Turner, 1938b; Burk, 1939; Marsh and Goddard, 1939; Merry and Goddard, 1941). The following formula was used to calculate Meyerhof quotients at the various O₂ tensions:

$$\text{Meyerhof quotient} = \frac{\text{c.mm. fermentation CO}_2 \text{ in N}_2 - \text{c.mm. fermentation CO}_2 \text{ at given O}_2 \text{ tension}}{\text{c.mm. respiration CO}_2 \text{ at same O}_2 \text{ tension}}$$

This method is essentially the same as that described by Dixon (1937) and Burk (1939).

Meyerhof quotients, shown in table 3, for seedlings at O₂ tensions above 0.3 per cent are from 1.5 to 2.0 for rice and 0.2 to 0.5 for wheat. The quotients for rice (comparable in magnitude to values reported for yeast) and those for wheat at certain O₂ tensions, together with the anaerobic CO₂/aerobic CO₂ ratios described, indicate that the Pasteur effect occurs in both seedlings and is of a very high degree in rice seedlings.

With increase in the O₂ tension, the values of the F/R ratio (ratio of fermentation to respiration where O₂ is present, 0.3 per cent or higher) range from 9 to 0 for wheat and from 53 to 0 for rice.

TABLE 4. *Respiration of excised embryos and endosperms at various O₂ tensions. All activities are expressed as cmm./hr./standard sample. R.Q.'s—Wheat: Total, 0.88; Embryo, 0.90; Endosperm, 0.82. Rice: Total 0.90; Embryo, 0.88; Endosperm, 0.96.*

Oxygen tension %	Total activities (Embryos and endosperms)				Embryo activities				Endosperm activities			
	Oxygen uptake	Total CO ₂ evolution	Resp. CO ₂ evol.	Ferm. CO ₂ evol.	Oxygen uptake	Total CO ₂ evolution	Resp. CO ₂ evol.	Ferm. CO ₂ evol.	Oxygen uptake	Total CO ₂ evolution	Resp. CO ₂ evol.	Ferm. CO ₂ evol.
<i>Wheat seedlings (Standard sample = 6.65 units)</i>												
20.8	124.0 ^a	97.5 ^a			100.0 ^a	77.5 ^a			24.0 ^a	20.0 ^a		
	99.5	97.5			71.0	71.5			28.5	26.0		
	116.5	105.0			93.0	88.0			23.5	17.0		
Ave.	113.0	100.0	100.0	0	88.0	79.0	79.0	0	25.5	21.0	21.0	0
10.4	77.0	117.5	69.0	48.5	52.5	88.5	47.2	41.3	24.5	29.0	21.1	7.9
5.2	53.5	79.0			41.0	53.5			12.5	25.5		
	59.0	86.0			44.0	63.0			15.0	23.0		
Ave.	56.3	82.5	50.0	32.5	42.5	59.0	38.5	20.5	13.8	24.0	11.5	12.5
2.6	27.5	74.0			19.0	61.5			8.5	12.5		
	22.5	75.5			15.0	59.0			7.5	16.5		
	20.5	67.5			16.5	56.5			4.0	11.0		
Ave.	23.5	72.5	21.0	51.5	17.0	59.0	15.3	43.7	6.6	13.5	5.4	8.1
0.7	20.0 ^b	...			15.5 ^b	...			4.5 ^b	...		
	13.0	52.0			8.0	38.5			5.0	13.5		
	14.5	67.0			9.5	45.5			5.0	21.5		
Ave.	15.8	59.5	14.0	45.5	11.0	42.0	9.9	32.1	4.8	17.5	3.9	13.6
0.3	3.5	63.0			2.5±	53.5			1.0±	9.5		
	1.5	71.5			1.0±	63.5			0.5±	8.0		
Ave.	2.5	67.5	2.2	65.3	1.7	58.5	1.5	57.0	0.8	8.8	0.7	8.3
0.0	0.0	70.5 ^c	0	70.5	0	57.0 ^c	0	57.0	0	13.5 ^c	0	13.5
<i>Rice seedlings (Standard sample = 12.50 units)</i>												
20.8	111.0	98.0			79.5	60.0			31.5	38.0		
	105.5	86.5			70.5	71.0			35.0	15.5		
	113.5	116.5			71.5	64.0			42.0	52.5		
Ave.	110.5	100.0	100.0	0	73.5	65.0	65.0	0	36.5	35.0	35.0	0
10.4	80.5	120.0	73.5	46.5	50.5	85.0	44.5	40.5	30.0	35.0	29.0	6.0
5.2	59.0 ^b	112.0			29.5 ^b	76.0			29.5 ^b	36.0		
	60.0	113.5			33.0	78.0			27.0	35.5		
	49.5	105.0			30.5	69.0			19.0	35.0		
Ave.	56.5	110.5	52.0	58.5	31.5	75.0	28.0	47.0	25.0	35.5	24.0	11.5
2.6	19.0	113.0			10.0	79.0			9.0	34.0		
	29.5	116.0			14.5	91.5			15.0	24.5		
	29.0	107.5			16.5	80.5			12.5	27.0		
Ave.	26.0	111.5	24.0	87.5	14.0	83.0	12.5	70.5	12.0	28.5	11.5	17.0
1.3	13.0	155.0			5.6	125.0			7.4	30.0		
	14.0	136.0			7.0	111.0			7.0	25.0		
	13.0	138.0			6.5	112.0			6.5	26.0		
Ave.	13.5	143.0	12.3	130.5	6.5	116.0	5.8	109.2	7.0	27.0	6.5	20.5
0.7	3.0	134.5			2.3	90.0			0.7	44.5		
	15.0	141.5			8.7	102.0			6.3	39.5		
	7.0	114.0			5.0	82.0			2.0	32.0		
Ave.	8.3	130.0	7.5	122.5	5.3	91.5	4.7	86.8	3.0	38.5	2.9	35.6
0.3	5.5	96.5			2.7	72.5			2.8	24.0		
	2.5	94.5			1.9	75.5			0.6	19.0		
	6.0	95.0			4.6	73.0			1.4	22.0		
Ave.	4.6	95.5	4.2	91.3	3.1	73.5	2.8	70.7	1.6	22.0	1.5	20.5
0.0	0	94.5 ^c	0	94.5	0	67.0 ^c	0	67.0	0	27.5 ^c	0	27.5

^a Each value represents the average of two tests, unless otherwise noted. ^b One test. ^c Three tests.

RESPIRATION OF EXCISED PARTS.—Having determined the respiration and fermentation of intact wheat and rice seedlings over a range of O_2 tensions, it seemed desirable to repeat this study on excised embryos (all of the intact seedling except the endosperm) and on endosperms, since the growth that was measured was that of the embryo, which occurred at the expense of food stored in the endosperm.

A technique almost identical with that employed for the intact seedlings was used for the separated parts. The only difference in the procedure was that the embryos were carefully dissected from the endosperms after the seedlings had been removed from the germinators, and then pretreatment was given to each of the excised parts of the seedlings.

The results of these excision tests are given in table 4 and figures 6 and 7. To facilitate comparison, the data are expressed in terms of seedling samples of such size that the sum of the CO_2 output of the excised embryos and endosperms is equal to 100 c.mm. per hour in air. This amount of activity was shown by 6.65 excised wheat seedlings and by 12.50 excised rice seedlings. All activities are expressed in terms of samples of the same size.

Since the standard samples for intact seedlings was 6.4 for wheat and 13.3 for rice, excision appears to have caused a 7 per cent decrease in the total CO_2 activity (embryo + endosperm) of wheat in air and a 6.5 per cent increase in that of rice seedlings in air. In response to excision, wheat O_2 uptake remained constant or increased very slightly, while excision resulted in a 10 per cent increase in O_2 uptake of rice.

In general the curves of figures 6 and 7 show the same trends as those of figure 5 for intact seedlings, and so it is evident that excision has produced no pronounced change in the over-all activity of the seedling parts.

From the curves of figure 6 and the values in table 4a for wheat it may be seen that approximately 80 per cent of the total (embryo + endosperm) activities are in the embryo over the entire range of O_2 tensions.

The striking difference between the respiratory quotient (R.Q.) of the excised embryo (1.0) and that of the endosperm (0.35) observed in barley by Merry and Goddard (1941) and noted in lower degree in endosperms (reduction to 0.5 or 0.6) in most of the tests conducted by Stoward (1908) was not found in wheat or rice in the present studies. For wheat, average values for the respiratory quotient in air were as follows: total, 0.88; embryo, 0.90; endosperm, 0.82. For rice they were: total, 0.90; embryo, 0.88; endosperm, 0.96. Enrichment of the substrate with sucrose, difference in temperature, or difference in the type of seedling and phase of development may have been controlling factors. The respiratory quotients for wheat and rice embryos in air are similar in magnitude to those noted in studies on barley (Stoward, 1908; James and James, 1940; Merry and Goddard, 1941).

The rate of CO_2 output by excised wheat endosperms and its proportion to total seedling CO_2 output in N_2 as well as in air were comparable to observations on barley endosperms by Barnell (1937); O_2 uptake by wheat endosperms was approximately the same (0.95 times as great) as that observed by Merry and Goddard in barley endosperms.

The relationships of fermentation and respiration to decrease in O_2 tension are essentially the same for the excised seedling parts and their total as for intact wheat seedlings.

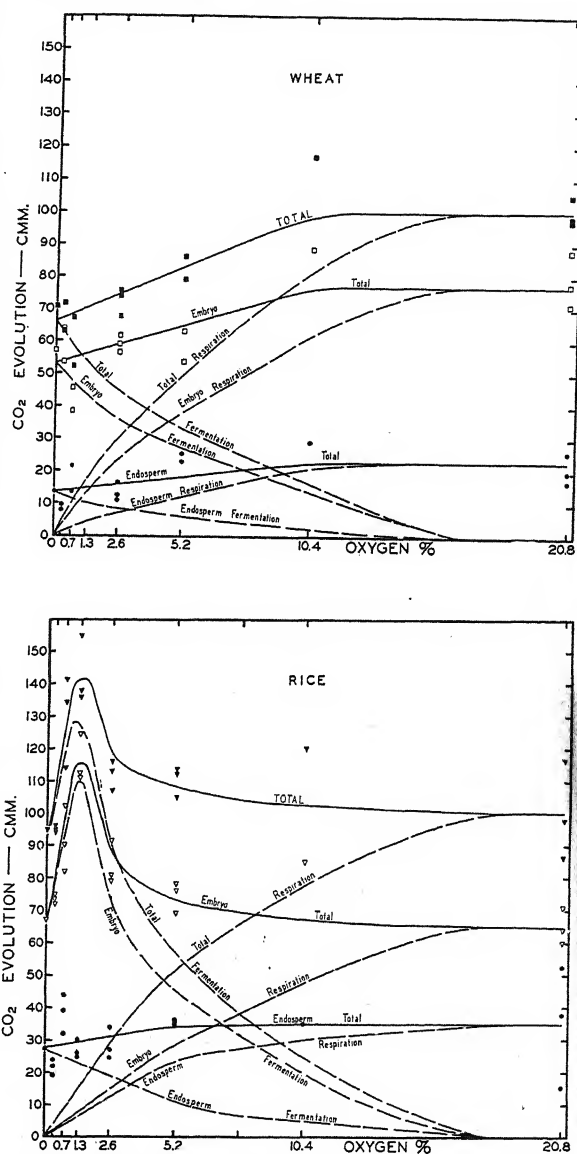


Fig. 6-7.—Fig. 6 (above). CO_2 evolution by excised wheat seedlings. Effects of O_2 tension on respiration CO_2 , fermentation CO_2 , and total CO_2 evolution of excised wheat germs (embryos) and endosperms.—Fig. 7 (below). CO_2 evolution by excised rice seedlings. Effects of O_2 tension on respiration CO_2 , fermentation CO_2 , and total CO_2 evolution of excised rice germs (embryos) and endosperms.

It may be seen from the curves of figure 7 (plotted from table 4) for excised embryos and endosperms of rice seedlings that in air the embryo activity of rice accounts for about 65 per cent of the total activity (embryo + endosperm), whereas the embryo activity of wheat (fig. 6) makes up about 80 per cent of the total activity. The rice embryo (0.75 mg.) makes up $1/18.5$ of the total weight of the seed, while the wheat embryo (1.65 mg.) makes up $1/15$ of the total weight of the seed. (These figures are averages for 1530 rice embryos and endosperms and 1050 wheat embryos and endosperms.) Therefore, when the relative proportions of the total seedling weight contributed by the excised embryos and endosperms are taken into consideration, it appears that the relation of embryo activity to total activity is the same in rice as in wheat ($65 \times 18.5/15 = 80$).

The distribution of respiratory activity observed in embryos and endosperms of wheat and rice agrees with that reported in wheat by a number of earlier investigators (see Lyon, 1928). The data of Stoward (1908) show that the embryo contributed a smaller fraction of the total CO_2 output of barley seedlings than was observed in these studies on wheat and rice. Since Stoward's data show a lower total CO_2 output, the difference could be due to lower activity in his barley embryos, respiration of which was measured in non-enriched substrate.

The response of the excised rice endosperms to decreasing O_2 tension was similar to that observed for wheat endosperms.

Both intact rice seedlings and excised rice seedlings showed a marked rise in CO_2 output as the O_2 tension decreased from 10 per cent to between 1.3 and 0.7 per cent. The form of the curve for total activity was determined mainly by embryo activity. The percentage of total activity carried on by the embryo increased from about 65 per cent at an O_2 tension of 10 per cent to approximately 85 per cent at an O_2 tension of 1.3 per cent.

Endosperm respiration gradually decreased to zero as the O_2 tension decreased to zero. Endosperm fermentation increased gradually as the O_2 tension decreased until, in N_2 , it amounted to about 30 per cent of the total CO_2 activity in air or in N_2 .

From the results thus far presented, we may conclude that at low O_2 tensions rice seedlings are superior to wheat seedlings in the degree to which they are capable of carrying on normal activities. With restricted O_2 supply, rice is more capable than wheat of germinating and maintaining growth. Rice can carry on these activities in very low O_2 concentrations, or even in the absence of O_2 , because it possesses a fermentation system which functions strongly enough to compensate for its decreased respiration. In contrast, wheat, which exhibits a stronger aerobic activity than rice at all O_2 tensions, is unable to germinate or grow at low O_2 tensions at rates nearly approaching those in air (a more nearly normal environment for wheat) because the increase in fermentation is not sufficient to compensate for

the reduction of respiration brought about by the decrease in the supply of O_2 .

The outstanding difference between the curves for excised seedlings of rice and those for intact seedlings is the fall in total CO_2 and fermentation CO_2 at O_2 tensions below 1.3 per cent. The total CO_2 represented chiefly embryo fermentation, and decreased markedly below an O_2 tension of 1.3. At an O_2 tension of 1.3 per cent the embryo produced 85 per cent of the CO_2 of fermentation and 80 per cent of the total CO_2 . In N_2 (zero O_2) the embryo produced 70 per cent of the CO_2 of fermentation and 70 per cent of the total CO_2 .

Below an O_2 tension of 1.3 per cent there is a conspicuous and apparently significant decrease in total CO_2 output, in total fermentation, in total embryo CO_2 output, and in embryo fermentation, while a slight but questionable increase occurs in endosperm CO_2 output.

Further experiments were conducted on intact rice seedlings in O_2 tensions below 1.3 per cent to check the results obtained (fig. 5, table 3). The results of these experiments gave no indication that a decrease, such as that observed in the CO_2 output of excised embryos, occurred in the activity of intact rice seedlings below an O_2 tension of 1.3 per cent. The reason for the decrease in total CO_2 output of excised rice embryos is not at once evident.

A possible explanation of the pronounced peak at an O_2 tension of 1.3 per cent in the curves for fermentation and total CO_2 output by rice embryo and seedling (fig. 7) may be formulated by considering the possibilities of the interaction of the toxicity of end-products of fermentation, the resynthesis of products of fermentation, and the Pasteur effect. It has been shown for rice (Sawa, 1903) and wheat (Karlsen, 1925) that fermentation end-products are toxic in various degrees. Meyerhof quotients and anaerobic CO_2 /aerobic CO_2 ratios indicate the operation of a strong Pasteur effect in rice. Resynthesis of fermentation products when O_2 was introduced into anaerobic systems which had high Meyerhof quotients was shown by Meyerhof (Meyerhof, 1925; see Burk, 1939).

The full potentialities of the fermentation system of excised rice embryos are not attained at O_2 tensions below 1.3 per cent because of retardation brought about by toxic products of the fermentation. As the O_2 tension rises from 0 per cent, increased respiration could oxidize some of the intermediate and end-products of fermentation.

Therefore, the combined effects of respiration and resynthesis could remove toxic fermentation products and allow fermentation, as indicated by CO_2 evolution, to progress more rapidly with increasing O_2 tension. However, at the same time the increasing O_2 tension produces a Pasteur effect (O_2 inhibition of the fermentative processes) which results in a decrease in CO_2 output by fermentation. The observed CO_2 curves could, therefore, be considered to represent the interaction of three effects of increasing O_2 ; the first two effects are initially strong-

est and decrease in strength, while the third effect is absent in pure N_2 and increases in strength as the O_2 tension rises. Above an O_2 tension of 1.3 per cent the Pasteur effect predominates and fermentation progressively decreases.

In calculating Meyerhof quotients, it is assumed that the fermentation going on in these seedlings at low O_2 tensions is of the alcoholic type. That alcoholic fermentation occurs in wheat and similar seedlings was shown by many of the earlier studies reviewed by Palladin (1926) and Kostychev (1927). More recently, the anaerobic activity in seedlings of cereal grains and various tissues of the higher plants has been shown, or sometimes merely assumed, to be alcoholic fermentation similar to that occurring in yeast (Haas and Hill, 1929; Kostychev, 1931; Dixon, 1937; Turner, 1937, 1938a, 1938b; Burk, 1939; Marsh and Goddard, 1939; Merry and Goddard, 1941). Most investigators have assumed that alcoholic fermentation is the predominant type of anaerobic respiratory activity in tissues of the higher plants. It should be borne in mind, however, that in plant tissues the ratio C_2H_5OH/CO_2 in fermentation does not always equal 1, and that other types of fermentation may be present. The recent investigations by Hohl and Joslyn (1941a, 1941b), as well as earlier studies on yeast fermentation in various media, have indicated that even in yeast—where alcohol is the main non-gaseous end-product of the fermentation—significant amounts of lactic acid or, to a lesser degree, formic acid are produced. It is evident that fermentation may involve a complex set of processes and may result in end-products of various types.

Since there is rather general agreement that alcoholic fermentation is typical of tissues of higher plants, and since it has been shown in many investigations that hexose enrichment of the substrate results in a C_2H_5OH/CO_2 ratio characteristic of alcoholic fermentation, it seems safe to consider that the fermentation occurring in the seedlings of this study, which received carbohydrate enriched substrates, was chiefly of the alcoholic type.

HEXOSE UTILIZATION.—The curves of figure 8, showing relative hexose utilization by intact seedlings, have been drawn from the smoothed curves of figure 5 by assuming that respiration is expressed by the equation $C_6H_{12}O_6 + 6 O_2 = 6 CO_2 + 6 H_2O$ and that fermentation is represented by the equation $C_6H_{12}O_6 = 2 CO_2 + 2 C_2H_5OH$. Relative rates of hexose consumption were, therefore, calculated by dividing respiration CO_2 (in c.mm. per hr.) by 6 and fermentation CO_2 by 2. All the curves represent the relative hexose destruction by a number of seedlings (wheat, 6.4; rice, 13.3) which in air would be capable of producing 100 c.mm. of CO_2 or of consuming 16.7 hexose units per hour.

With decrease in O_2 tension, consumption of hexose increases in both types of seedlings, but the change is much more pronounced in rice than in wheat. At O_2 tensions below 5.2 per cent, intact rice seedlings use up respiratory reserves from 3.5 to 4.6

times as rapidly as in air, while wheat seedlings (in which fermentation is relatively weak) use up their reserves about 1.7 times as rapidly as in air.

A marked Pasteur effect (or O_2 inhibition of fermentative processes) is indicated for rice and a much weaker effect for wheat. Dixon (1937) and Burk (1939) have presented excellent discussions of the Pasteur effect, which involves the action of O_2 in diminishing the rate of carbohydrate destruc-

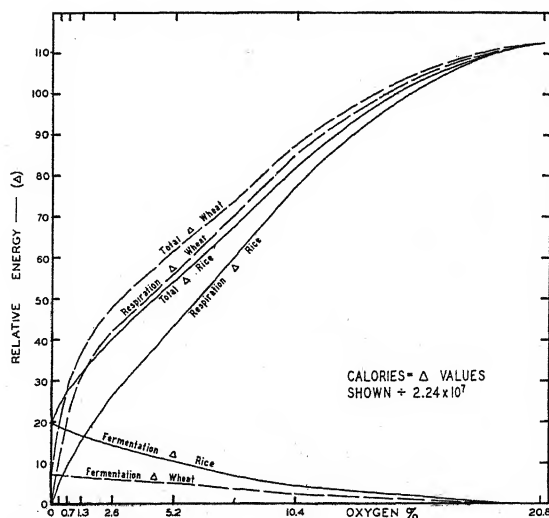
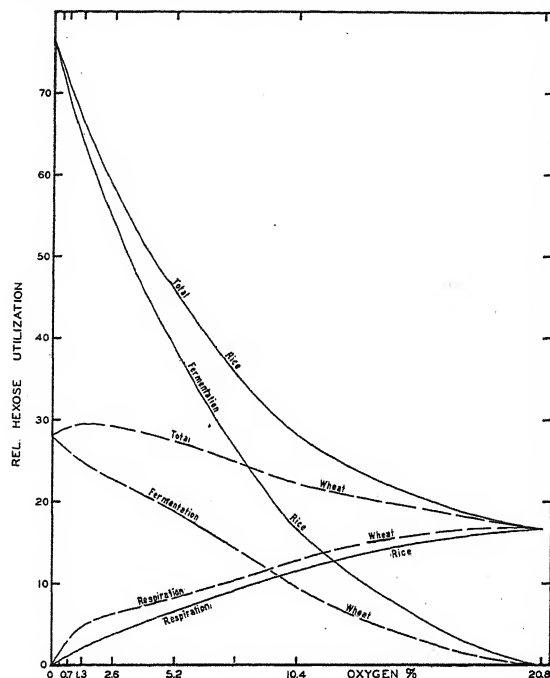


Fig. 8-9.—Fig. 8 (above). The relation between O_2 tension and relative hexose utilization by intact wheat and rice seedlings (calculated from CO_2 evolution).—Fig. 9 (below). The effect of O_2 tension on the energy released by intact wheat and rice seedlings (calculated from CO_2 evolution).

tion and in suppressing the accumulation of alcohol or other products of fermentation. When the Pasteur effect operates, introduction of O_2 into an anaerobic system may be regarded as preserving hexose reserves that would be destroyed in the absence of O_2 . The number of hexose molecules thus conserved per hexose molecule consumed in respiration may be calculated from values read from the curves of figure 8 by means of the following computation: The total hexose used at any O_2 tension is subtracted from the total hexose used under anaerobic conditions, and the resulting difference (representing the total saving in hexose) is divided by the hexose used in respiration at the same O_2 tension. (The same result is obtained by a computation from the Meyerhof quotient, previously defined, by the use of the following formula: $3 \times M.Q. - 1.0$.) Indices of the Pasteur effect on hexose conservation, calculated in this manner, are summarized in the following tabulation:

O_2 tension	Hexose molecules preserved per hexose molecule used in respiration	
	In rice	In wheat
0.3	4.9	0.3
0.7	4.2	(-0.3)
1.3	4.5	(-0.2)
2.6	4.7	(-0.2)
5.2	4.8	0.1
10.4	4.2	0.4
20.8	3.6	0.7

It is evident that rice seedlings exhibit a pronounced Pasteur effect. For every hexose molecule used in respiration approximately 4 hexose molecules that would be destroyed in the absence of O_2 are preserved as respirable substrate. In contrast, wheat seedlings show little or no conservation of hexose in the presence of O_2 . Although these results, based upon gas exchange, strongly indicate the operation of the Pasteur effect in rice seedlings, it would be desirable to verify them by means of direct measurements of loss of carbohydrate.

In addition to serving as respiratory substrate, sugar is utilized in the growth of the seedling embryo. In this connection we may note the results obtained by Barnell (1937) and by Terroine, Bonnet, and Joessel (1924) on the "utilization coefficients" of barley and rice growing in air. Barnell found that in air approximately 64 per cent of the total hexose made available to the embryo by translocation from the endosperm was used in growth by barley embryos, and Terroine *et al.* reported that about 60 per cent was used by rice seedlings. The latter workers also reported that in air little or no difference was to be found between seeds having endosperms of similar chemical composition; and, in regard to the chemical nature of seed reserves, they stated that starchy seeds had relatively low utilization coefficients.

ENERGY RELATIONSHIPS.—The influence of O_2 tension on the energy released in fermentation and

respiration is shown in the graphs of figure 9. These were prepared from the data of figure 8 by multiplying the hexose consumed in respiration by 6.74 and that consumed in fermentation by 0.26, these figures representing the number of kilogram-calories of heat energy liberated by 0.01 gram-molecule of hexose (Kostychev, 1927; Haas and Hill, 1929). As in the preceding figure, all the curves of figure 9 show the activity of standard samples of intact seedlings (6.4 wheat seedlings, 13.3 rice seedlings). In air these samples would be able to produce 100 c.mm. of CO_2 per hour, or consume 16.7 hexose units, or release 112.4 energy units.

With a reduction in O_2 tension, there is a rapid decrease in rate of total energy liberation, so that under completely anaerobic conditions the rate in rice is $1/6$ that in air and the rate in wheat is $1/15$ that in air. It may be seen that as the O_2 tension is decreased, the total energy released by wheat remains greater than that by rice until an O_2 tension of 0.7 per cent is reached; below this, the total energy of rice drops less rapidly than that of wheat. Fermentation energy becomes greater than respiration energy in rice below an O_2 tension of 1.3 per cent and in wheat below an O_2 tension of 0.3 per cent.

Evidence from many sources points to the conclusion that release of energy is necessary in order that growth may occur. This relationship is indicated by the fact that the decrease in growth of both seedlings roughly paralleled the decrease in total energy liberated in respiration and fermentation. The correlation between growth and energy release is much closer in wheat than in rice. With reduction in O_2 tension, growth of rice remains relatively rapid in spite of a marked decrease in the rate of total energy liberation. Wheat sets free total energy more rapidly than rice above an O_2 tension of 0.7 per cent, and yet even at 5.2 per cent O_2 its growth rate (relative to that in air) is much lower than that of rice.

Throughout the range of low O_2 tensions, where rice exhibits greater growth than wheat, it also liberates more energy in fermentation. These relationships suggest the possibility that, in promoting growth, the energy released anaerobically in fermentation is more efficiently used than the energy liberated in oxidative respiration.

RELATION OF ALCOHOL TO GROWTH AND RESPIRATION.—The experiments described in the preceding sections of this paper show that rice seedlings exhibit a functional fermentation system about 2.5 times as strong as that shown by wheat seedlings and at low O_2 tensions rice seedlings accomplish approximately twice as much growth as wheat seedlings (growth is expressed as percentage of the normal growth made in air). Nevertheless, the possibility exists that the fermentation system may be potentially as strong in wheat seedlings as in rice seedlings. The greater activity of rice than of wheat could result if the fermentation systems of the two

TABLE 5. *Respiratory and growth activities of intact wheat and rice seedlings in various alcohol concentrations.*

Oxygen tension %	Ethyl alcohol ^b cc.	Tissue	Respiratory activity ^a			Growth activity		
			CO ₂ evolution %	O ₂ uptake %	R. Q. %	Increment embryo ^d %	Root ^e %	Shoot ^e %
20.8	0 ^c	Wheat	100	100	100	100	37.5	62.5
		Rice	100	100	100	100	32.0	63.5
	0.5	Wheat	20.5	7.5	11.0
		Rice	68.0	18.0	48.0
	1.0	Wheat	8.5	4.0	3.0
		Rice	32.0	3.0	23.0
	2.0	Wheat	3.0	1.5	1.0
		Rice	9.0	1.5	3.5
	3.0	Wheat	89	90	99
		Rice	86	92	93
	4.0	Wheat	+	+	+
		Rice	4.5	0	2.5
	5.0	Wheat	75	78	96	0	0	0
			83	88	94			
		Rice	80	82	95	0	0	0
			77	98	80			
	6.0	Wheat	74	76	97
		Rice	83	90	92
1.3	6.0 ^c	Wheat
		Rice	99	78	126

^a Each value represents the average of two tests.^b Cc. of absolute alcohol in 100 cc. of solution or buffered sucrose substrate.^c Control respiratory values (100%) are the average of twelve tests. Absolute control values (cmm./hr./mg. dry embryo): Wheat: CO₂, 9.90; O₂, 10.10; R.Q., 0.98. Rice: CO₂, 9.80; O₂, 9.85; R.Q., 1.00.^d Averages of three tests (45 units).^e Averages of one test (15 units). 100% relative to all growth data represents absolute increment of embryo dry weight, 12th to 120th hour, in water (wheat, 10.7 mgs.; rice, 2.2 mgs.).^f Absolute control values (100% cmm./hr./mg. dry embryo: CO₂, 12.35; O₂, 1.80; R.Q., 6.90).

types of seedlings were potentially equal, but if other factors, such as accumulation of toxic metabolic products and difference in degree of tolerance of products in the two seedlings, operated in such a manner as to allow a higher functional respiratory metabolism in rice seedlings than in wheat seedlings.

It has been noted that alcohol is produced by higher plants when they are supplied with a hexose substrate under anaerobic conditions. The production of alcohol by rice was established by Takahashi (1903, 1905), and studies by Sawa (1903) and Nagai (1916) have shown that rice seedlings are capable of growth on a substrate containing dilute ethyl alcohol. Karlsen (1925) found that ethyl alcohol in low concentration inhibited both fermentation and respiration in wheat seedlings, and Gray (1941) noted that different strains of yeast vary in their alcohol tolerance and differ with regard to the minimum concentration of alcohol needed to inhibit fermentation.

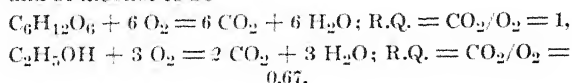
Ludwig *et al.* (1941) have recently confirmed the finding that alcohol is produced anaerobically by tissues of higher plants similar to those employed in the present studies and that, when hexose substrate

is supplied, CO₂ and C₂H₅OH are produced approximately according to the principal reaction in alcoholic fermentation of hexose. By means of manometric and chemical tests, they also showed utilization of alcohol as a respiratory substrate by roots of various legumes, oats, and maize.

In an attempt to determine whether or not differences exist between wheat and rice seedlings with respect to alcohol tolerance and utilization, tests of growth and respiration were conducted with alcohol supplied to the seedlings in various concentrations. A summary of these experiments appears in table 5. The results indicate a slight superiority of rice seedlings over wheat seedlings in ability to grow in alcohol solutions containing up to 2 cc. of ethyl alcohol in 100 cc. of solution. Comparing root and shoot reaction in the seedlings, it is seen that rice shoots showed greatest tolerance of alcohol, making most nearly normal growth increment. Rice roots were less tolerant of alcohol than rice shoots but more tolerant than shoots or roots of wheat.

The data on respiration are not extensive enough to warrant treatment comparable in detail to that used by Ludwig *et al.* However, considering the re-

actions for the complete aerobic oxidation of hexose and of alcohol to be



a decrease in the R.Q. of aerobic respiration below unity, when alcohol was present in the substrate in addition to hexose, would strongly suggest that alcohol, as well as hexose, was being utilized as oxidation substrate.

From table 5 it may be seen that both wheat and rice gave indications of a small progressive decrease in the R.Q. as alcohol was added to the medium in increasing amounts. The decrease in R.Q. was not so great as that reported in the more extensive studies of Ludwig *et al.* In the latter studies the decrease in O_2 uptake was much less than the decrease in CO_2 output when alcohol was added in increasing amounts; this indicated a relatively high tolerance of the oxidation system for alcohol. In the tests on wheat and rice seedlings supplied with alcohol, the reduction in CO_2 output was only slightly greater than the reduction in O_2 uptake, and so the R.Q.'s of wheat and rice seedlings did not show decreases comparable in degree to those observed by Ludwig *et al.* in roots of oats and corn, and they did not indicate so much alcohol oxidation. Differences in concentration of hexose in substrate, length of test period, methods, and tissue could account for the difference in degree of reduction of the R.Q. On the basis of the present tests, it appears that the respiratory activities of wheat and rice seedlings respond in similar degree to alcohol, while the growth of the seedlings indicates a slightly higher tolerance of alcohol by rice than by wheat. This relationship could favor a higher functional activity of the fermentation system in rice than in wheat.

SUMMARY

Comparative studies of growth and respiratory activities in seedlings of winter wheat (*Triticum vulgare*) and of lowland rice (*Oryza sativa*) were made at O_2 tensions ranging from 0 to 20.8 per cent. The rate and extent of germination were recorded over a 72-hour period, and growth of seedlings initially 12 hours old was determined for a 96-hour period; in these tests the seedlings were kept on moist filter paper in chambers continuously flushed with gas. Manometric measurements of the gas exchange occurring in seedlings with roots 6 mm. long were conducted according to the technique of Dickens and Simer. The seedlings were provided with a phosphate buffer (pH 5.8) containing 0.117 molar sucrose.

In the absence of O_2 the germination of rice seeds was reduced less than 10 per cent below that in air and was accomplished at more than half the normal rate. No germination of wheat occurred under similar conditions. Significant reduction in the extent and rate of germination of wheat occurred when the O_2 tension was lowered to 5 per cent, and consider-

ably less than half of normal germination resulted in O_2 concentrations below 1 per cent.

At O_2 tensions of 5 per cent or less, rice seedlings made approximately twice as much growth as wheat seedlings (on the basis of increment in dry weight of embryo in air). Reduction in O_2 tension inhibited roots of both seedlings more than shoots, and it had least effect on rice shoots.

The results of the manometric measurements of gas exchange showed that when the respiration rate was expressed in terms of CO_2 evolved per mg. of dry weight of embryo per hour, intact rice seedlings had 87 per cent as great activity in air as wheat seedlings. Per seedling, however, the CO_2 output of rice in air was 48 per cent as rapid as that of wheat in air.

With decrease in O_2 tension from 20.8 to 0 per cent, the rate of O_2 uptake decreased similarly in both kinds of seedlings, but it remained higher in wheat than in rice over the range of O_2 concentrations.

Oxygen uptake of both seedlings was partially inhibited by sodium azide and carbon monoxide, thus indicating at least partial dependence upon cytochrome oxidase.

In the complete absence of O_2 , rice seedlings evolved CO_2 1.5 times as rapidly as in air, whereas wheat seedlings under similar conditions gave off CO_2 only about 50 per cent as rapidly as in air.

It may be concluded that the superiority of rice over wheat in ability to germinate and grow in very low O_2 concentrations is dependent upon the possession by rice of a highly functional fermentation system that more than compensates for a respiration system that is even weaker than that of wheat.

Meyerhof quotients calculated from the gas exchange over the range of O_2 tensions were from 1.5 to 2.0 for rice seedlings and from 0.2 to 0.5 for wheat seedlings. These quotients pointed to a strong Pasteur effect in rice and a very weak effect in wheat. Calculations indicated that for every hexose molecule used in respiration by rice seedlings, approximately 4 hexose molecules that would have been destroyed in the absence of O_2 were preserved as respirable substrate.

With reduction in O_2 tension there appeared to be a rapid decrease in the rate of total energy liberation, so that under completely anaerobic conditions the rate for rice was 1/6 that in air and the rate for wheat was 1/15 that in air.

A consideration of gas exchange indicates that in the range of low O_2 concentrations, where rice exhibited greater germination and growth than wheat, it also liberated more energy in fermentation. The possibility is suggested that, in promoting growth, energy released anaerobically in fermentation may be more efficiently used than the energy liberated in oxidative respiration.

In studies of excised parts of wheat and rice seedlings, it was found that from 65 to 85 per cent of the total CO_2 output at any O_2 tension was contributed

by the embryo. The endosperm in rice made up a larger fraction of the weight of the seed than in wheat, and it accounted for a correspondingly greater percentage of the total CO_2 evolution.

Growth studies showed that rice seedlings were more tolerant of alcohol than wheat seedlings. Some utilization of alcohol as a substrate for aerobic res-

piration was observed in both seedlings. Some of the implications as to substrate utilization, energy release and utilization, and effects of metabolic products are briefly discussed.

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VASCULAR DIFFERENTIATION IN THE VEGETATIVE SHOOT OF LINUM. I. THE PROCAMBIUM¹

Katherine Esau

THE PRESENT paper is the first of two articles dealing with the vascularization of the vegetative shoot apex of *Linum perenne* L. Although the course of development of the first xylem in the shoots of seed plants is rather well understood, the information on the pattern of differentiation of the procambium and the first phloem is very scanty (see Esau, 1938, 1939, 1943). The origin of the first vascular meristem in the shoot apex is receiving some attention from workers who are concerned with the organization of the apical meristem and with foliar histogenesis (Boke, 1940, 1941; Cross, 1942; Cross and Johnson, 1941; Satina and Blakeslee, 1941). Then there are studies which attempt to analyze the delimitation of the vascular system in relation to the early differentiation of the axis as a whole (Helm, 1931; Louis, 1935; Kaplan, 1937). Finally, Priestley and his co-workers (Priestley and Scott, 1936, 1937; Priestley, Scott, and Gillett, 1935; Scott and Priestley, 1925; Smith, 1941) are considering vascular development with reference to the phyllotaxy of the plant. These various methods of approach to the study of vascular initiation will have to be coordinated before a complete and unified picture of vascularization of shoots can emerge. The present study is an attempt at such a coordination. In particular it is concerned with the comparative patterns of initial development of the

procambium, the xylem, and the phloem, and with the relation of the course of vascularization to the phyllotactic pattern of the plant.

MATERIAL AND METHODS.—*Linum perenne* was chosen for this study because of its relatively simple vascular system. This species has only one trace per leaf and very few axillary buds. The simple structure of the plant proved particularly advantageous in the determination of the longitudinal course of development of the first vascular elements—the subject to be considered in the second article of the series. For the study of procambial differentiation, however, more suitable material than flax could have been chosen. Generally, plants with opposite leaves are most convenient for studies of the histogenesis at shoot apices because of the relative ease with which the shoots of such plants can be oriented on the microtome to give median sections of the youngest primordia. The flax has alternate leaves which are considerably crowded. The cells of the *Linum perenne* shoot apices are relatively small, another disadvantage in the study of histogenetic details.

The shoots were collected at different times during the spring and summer from several plants growing outdoors. The slides were prepared by the ordinary paraffin method, with normal butyl alcohol as a paraffin solvent and a paraffin-beeswax-rubber mixture as embedding medium. Chrom-acetic-formalin and

¹ Received for publication June 18, 1942.

formalin-acetic-alcohol mixtures were employed for killing and fixing. All the photomicrographs were taken from material treated with the chrom-acetic-formalin solution. The sections which were cut 6 to 7 microns thick were stained with a combination of iron-alum-hematoxylin and safranin according to a schedule previously indicated by the writer (Esau, 1941). The principal feature of this schedule is that the hematoxylin is used in a very dilute solution and is not allowed to overstain the sections. The method is very simple and gives good wall differentiation.

THE GENERAL CHARACTERISTICS OF THE PROCAMBIUM.—The small leaves are arranged according to variable phyllotactic patterns on the slender stem of *Linum perenne*. The phyllotaxy and the organization of the vascular system of this plant will be considered in the second paper. Here it will suffice to indicate that each leaf has a single trace and that the different traces anastomose within the stem in a characteristic manner, so as to form a network. In transverse sections the network appears resolved into discrete units—the vascular bundles (fig. 12).

As is well known, the precursor of the primary vascular system is a meristem which is commonly referred to as *procambium*.² The procambium of the flax stem, like that of other plants, forms a network similar to that of the mature vascular system. Since, however, the procambial network is delimited before the internodes elongate, all its parts are very short. Largely through elongation of cells the procambial strands keep pace with the elongation of the internodes.

A superficial examination of shoot-apex sections indicates that the procambium occurs very close to the apical meristem (fig. 1). It also shows that the typical narrow elongated shape of the procambial cells is acquired gradually, the cells nearest the apex being the shortest (fig. 1-3). The density of the cytoplasm of the young procambium cells is a very striking feature (fig. 1-4, 11, and 22), and in stained sections the procambium strands frequently appear darker than the youngest cells at the apex of the shoot (fig. 1; compare also figures 4 and 5). Perhaps the procambial cells have actually somewhat denser protoplasts than the cells at the apex, but two other circumstances are, no doubt, largely responsible for the characteristic appearance of the young procambial strands. Firstly, they are flanked by the considerably vacuolated parenchyma cells of the pith and cortex, and the light staining of these cells emphasizes the darkness of the procambium. Secondly, the procambial cells have small transverse diameters; they are even smaller than those of the cells at the very apex (compare procambial cells indicated by arrows in figures 5 and 10 with the apical-meristem cells in figures 5 and 6, respectively), so that in a given depth of a longitudinal section more cells occur in the procambium than in the adjacent parenchyma. Still another feature of the procambium often con-

tributes to its dense appearance: in sections fixed less satisfactorily than usual, procambial protoplasts are considerably more shrunken than those of the parenchyma about them.

Incidentally, the procambium is not the only tissue that appears denser than the apical meristem. The leaf primordia which are, essentially, areas of active cell multiplication, stain very deeply (fig. 1-5, and 8). Furthermore, dense staining is evident in the peripheral part of the axis just below the apex (fig. 1 and 5), the part where the divisions occur that are concerned with the lateral expansion of the axis and the initiation of leaf primordia.

In older regions of the stem the procambial cells undergo a pronounced vacuolation (see the central parts of the bundles in figure 29) until the procambium becomes as highly vacuolated as the cambium.

THE DELIMITATION OF THE VASCULAR REGION IN THE SHOOT APEX.—The literature pertaining to the phenomena of the first delimitation of the vascular region in shoot apices is being reviewed in detail in a special article (Esau, 1943). Only the most important of these works are considered here again and only very briefly. According to Louis (1935), the vacuolation³ and enlargement of the cortical and pith cells bring about a delimitation of the meristem *prodesmogen* within which the vascular system is eventually differentiated. Helm (1931) suggested the term *primary meristem ring* for the thus delimited precursor of the vascular system of the axis, because in transverse sections of the latter the meristem appears as a ring. Kaplan (1937) emphasized the variability in shape of this meristematic region in different types of plants and organs and proposed the term *residual meristem* for it, as well as for all other regions which remain in a meristematic state below the apex, after certain other tissues of the same level have ceased to function as meristems. The term *residual meristem* implies that the meristem is a remainder of the apical meristem. Helm (1931) and Louis (1935) agree with Kaplan that, when first delimited, the precursor of the vascular system is a remnant of the apical meristem; in fact, all three workers stress the similarity of this meristem with the apical meristem. It is delimited because the adjacent tissues become modified and not because it itself changes. This meristem is not yet procambium: the remainder of the apical meristem undergoes certain characteristic changes before it becomes procambium.

Louis (1935) points out that the blocking out of the prodesmogen does not occur above the youngest leaf primordia. On the contrary, the delimitation of the vascular system of the axis occurs in an intimate relation with the differentiation of the leaves. The base of the leaf, the so-called "foliar buttress," constitutes a part of the axis. Hence, when the prodes-

² The writer prefers *procambium* to the recently introduced *provascular tissue* or *provascular meristem*. The reasons for this preference will be considered in a forthcoming review article (Esau, 1943).

³ Since all cells, including those of the apical meristem, are more or less vacuolated (Zirkle, 1932; Foster, 1942), statements in this paper, to the effect that one tissue vacuolates and some other does not, mean that at a given stage of development of the axis one tissue is more conspicuously vacuolated than another. For convenience of expression the comparative form is seldom used.

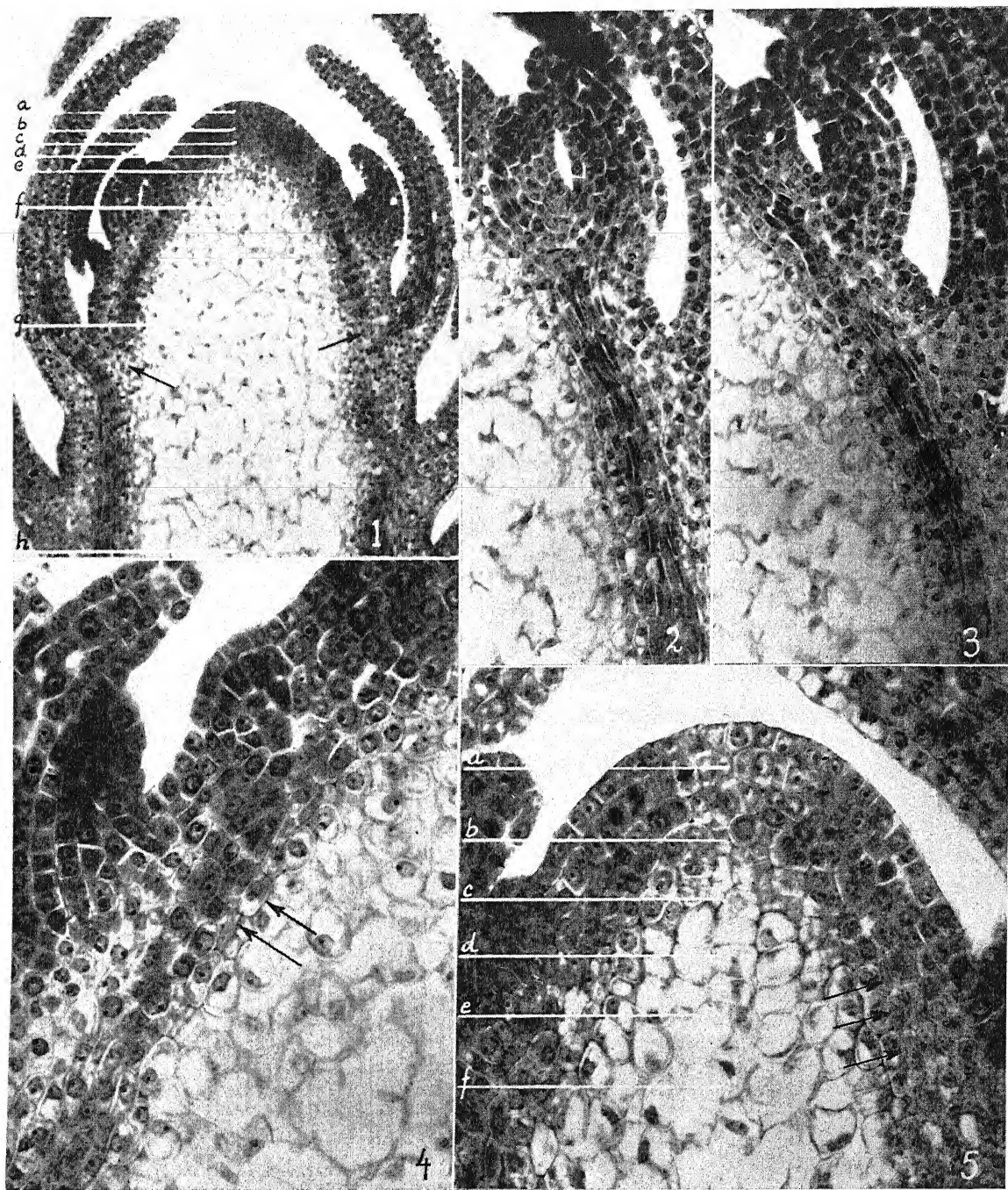


Fig. 1-5. Longitudinal sections of shoot apices.—Fig. 1 and 5. Median sections of shoot apices showing the apical meristem and leaves in different stages of development. The white lines at *a* to *h* indicate the approximate levels of figures 6 to 12 and 26, respectively. $\times 120$ and $\times 500$.—Fig. 2 and 3. Two successive sections 7 microns apart, showing the trace procambium of a young primordium. $\times 280$.—Fig. 4. A similar section as in figure 3 but of a younger primordium. $\times 500$.

mogen becomes delimited in the leaf primordium and its buttress, it is also blocked out in the axis. In the large-leaved dicotyledons the parenchymatous differentiation of the cortex and pith occur approximately at the same level, so that the prodesmogen

early assumes a ring-like form in transverse sections of the stem. The medullation of the axis of gymnosperms and small-leaved angiosperms typically begins higher than the parenchymatization of the cortex (Louis, 1935; Kaplan, 1937), and, consequently,

the future vascular region is at first not set off from the cortex, the two together appearing as a "peripheral meristem" (Kaplan, 1937).

Linum perenne is an example of a small-leaved dicotyledon in which the vacuolation of the pith is evident at a higher level than that of the cortex (fig. 1, 5, and 6-12). Comparatively uniform and fairly dense cells occur through about the first 30 microns of the apex (fig. 6; 1 and 5 at *a*). Farther below, the vacuolation of the pith cells sets in, but the peripheral region, where the cortex and vascular tissues will differentiate, remains densely cytoplasmic (fig. 7; 1 and 5 at *b*). The leaf primordia are initiated at this level (fig. 7, 13, and 15). The contrast between the lightly-staining vacuolated pith and the peripheral-meristem region is very striking about 50 microns below the apex, where the pith cells show also considerable enlargement (fig. 8; 1 and 5 at *c*). The differentiation of the pith progresses very rapidly. A short rib meristem (fig. 1 at *d*; 5 between *b* and *d*) is succeeded by the young pith in which intercellular spaces appear (fig. 1, 5, 9, and 10). Still farther below, the pith disintegrates (fig. 1, 11, and 12). The level at which the pith breaks down varies somewhat in shoots collected during different times of the season, the examples shown here representing types with rapidly disintegrating pith.

Louis (1935) would say that the prodesmogen constitutes the inner portion of the peripheral meristem at levels shown in figures 8 and 9. Eventually the parenchymatization of the cortex would occur and delimit the prodesmogen on the outer side. The early stage of cortical vacuolation in flax is slightly indicated in figure 10. In the axils of some of the primordia evident in this figure the protoplasm of the parenchyma appears somewhat lighter than that of the primordium itself and of the subjacent layer of tissue (fig. 10, white arrows). In the words of Louis (1935), the ventral parenchymatization of the foliar buttresses has set in. In figure 11 the parenchymatous differentiation has involved also the dorsal sides of the buttresses, and the cortex is, therefore, defined around the entire axis. The vascular region is now clearly delimited. It is, at this stage of development, not a homogeneous meristematic cylinder but is composed of discrete procambial strands alternating with the comparatively highly vacuolated interfascicular areas. Thus the vascular system, when first completely delimited by the vacuolation of the pith and the cortex, is past the stage of prodesmogen in Louis' (1935) sense. Neither is there any evidence at higher levels of a region that could be interpreted as prodesmogen (Louis, 1935) or primary meristem ring (Helm, 1931). A careful comparison of transverse and longitudinal sections shows that processes initiating procambium may be followed to the highest levels of the axis at which leaf primordia are being formed. Before the vascular system is finally delimited, procambial differentiation is indicated by active longitudinal divisions in localized areas of the inner part of the peripheral meristem (fig. 10 and 21). Divisions occur throughout the peripheral meri-

stem, but are obviously more numerous in these areas. Strands of narrow cells, the procambial strands, result from the localized divisions (fig. 11 and 22). At the upper levels of the axis (fig. 8 and 9) the youngest procambial cells are not obvious. The rows of these cells converge toward the apex and, therefore, in transverse sections, the procambial strands are cut on a bias and the relatively small diameters of their cells are obscured. On the other hand, at the highest levels of procambial differentiation, the cytoplasm of the procambium is not yet strikingly different in its density from that of the other cells in the peripheral region. Longitudinal sections, however, indicate that divisions leading to procambial differentiation occur at these levels (fig. 13 and 15).

Thus the procambial divisions are evident in connection with the youngest leaf primordia, that is, directly below the apical meristem (fig. 13 and 15), and no meristem is interpolated between the apex and the procambium that would merit a special designation as prodesmogen or meristem ring. The vascular region begins its existence at the apex not as a homogeneous meristem, not as a remainder of the apical meristem, but as a region in which certain groups of cells are in the process of being differentiated as procambial cells.

THE LONGITUDINAL COURSE OF DEVELOPMENT OF THE PROCAMBIUM.—According to Louis (1935), the prodesmogen of the leaf and axis is blocked out as a unit. Furthermore, the prodesmogens of the successive leaf buttresses are confluent, so that the vascular system of the axis is from its inception a continuous structure. Although Louis (1935) did not consider the phenomenon of procambization, his paper indicates that he regarded the delimitation of the prodesmogen and the differentiation of the procambium as two distinct phenomena (see review by Esau, 1943). The initial unity of the prodesmogen did not, therefore, imply unity of the procambium. Many workers hold the view that the procambium first appears at the base of the leaf and thence differentiates acropetally into the elongating leaf and basipetally into the axis (see review by Esau, 1943). Recently, however, students of apical meristems have begun to bring forth evidence that the procambium differentiates acropetally in complete continuity from the axis into the leaves (Boke, 1940, 1941; Satina and Blakeslee, 1941); or that at least it is continuous at all times in a given internode and leaf (Cross and Johnson, 1941; Cross, 1942).

The study of the course of procambial development is difficult because of several structural features. Firstly, the transition from the embryonic cells characteristic of the apex to the narrow elongated cells of the procambium is very gradual and one must decide somewhat arbitrarily what cells should be interpreted as the first procambial cells. Secondly, the procambium is apparent in connection with the youngest leaf primordia in the portion of the axis where the internodes, and with them the parts of the procambial system, are still extremely

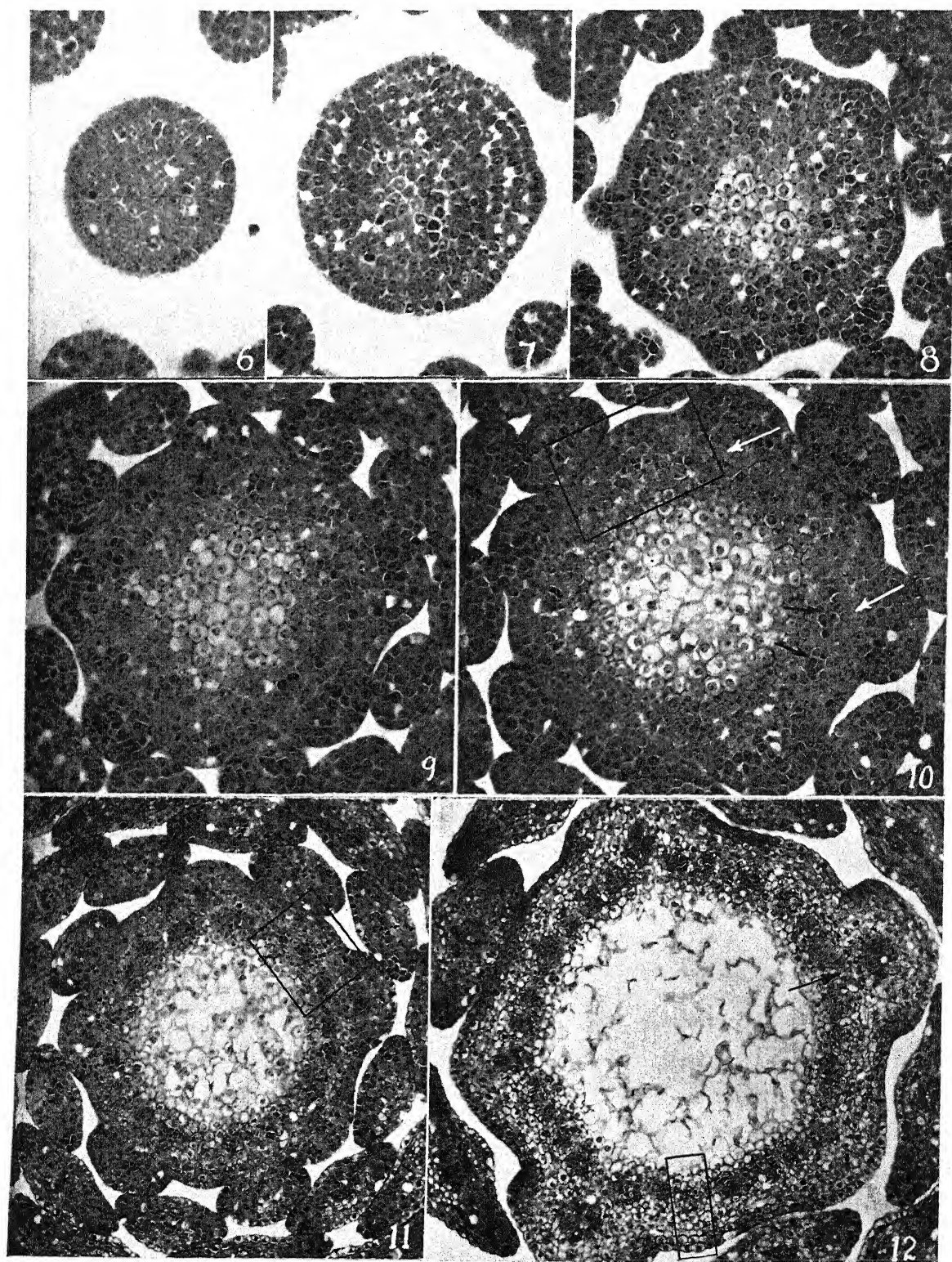


Fig. 6-12. Transverse sections of a shoot taken, respectively, the following number of microns below the apex: 12, 36, 54, 72, 90, 132, 294. The approximate positions of these sections are indicated in the longitudinal sections in figures 1 and 5 at *a* to *g*, respectively. The rectangles in figures 10 to 12 indicate the areas which appear more highly magnified in figures 21, 22, and 24, respectively. Fig. 6-10, $\times 250$. Fig. 11 and 12, $\times 160$.

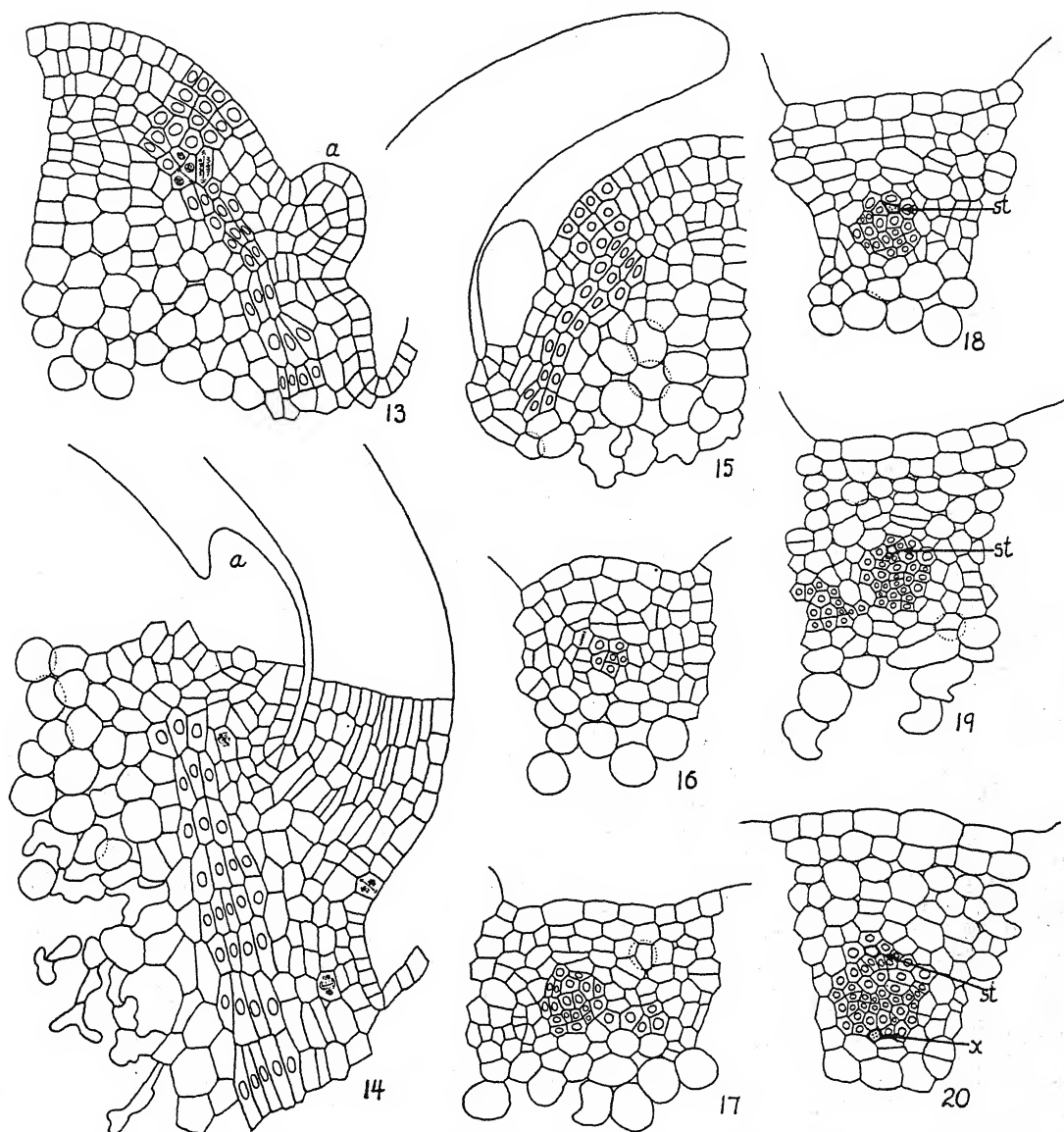


Fig. 13-20.—Fig. 13-15. Longitudinal sections of shoot apices illustrating early stages in the organization of the trace procambium. Nuclei have been drawn in the cells concerned with the formation of new leaf primordia and of the procambium. Figures 13 and 14 represent two sections 14 microns apart from the same apex; the former shows the upper, the latter the lower part of the trace of the youngest primordium. The use of two sections was necessary because in figure 13 the trace terminated above the gap of a lower leaf. For proper orientation regarding levels one leaf has been marked with an *a* in both views.—Fig. 16-20. Transverse sections of stem showing differentiation of trace procambium. Nuclei have been drawn in procambial cells only; *st*, sieve tube; *x*, xylem element. The sections were taken the following number of microns below the apex, respectively: 98, 140, 245, 315, 385. The leaves whose traces appear in figures 16, 17, and 18 were 21, 70, and 266 microns high, respectively; in figures 19 and 20, over 400 microns high. All figures $\times 397$.

short, so that the course of the individual bundles is not readily followed. Thirdly, the procambial strand of a given leaf differentiates in an oblique manner above the gap associated with the attachment of the next lower leaf located on the same vertical line as the first leaf. (Gaps are indicated with arrows in fig. 1 and 12.) If the procambium is followed in successive transverse sections, the identity of this meristem is easily lost where the strand, because of its oblique

course, is cut on a slant. A clear understanding of the phyllotaxy and interconnection of traces of the given plant helps to reduce this difficulty. The continuity of procambium in the gap region is more readily established in longitudinal than in transverse sections. A median longitudinal section through a leaf primordium would, of course, show an apparent break in the continuity in the procambium strand directly above the gap parenchyma (fig. 2). But a section which is

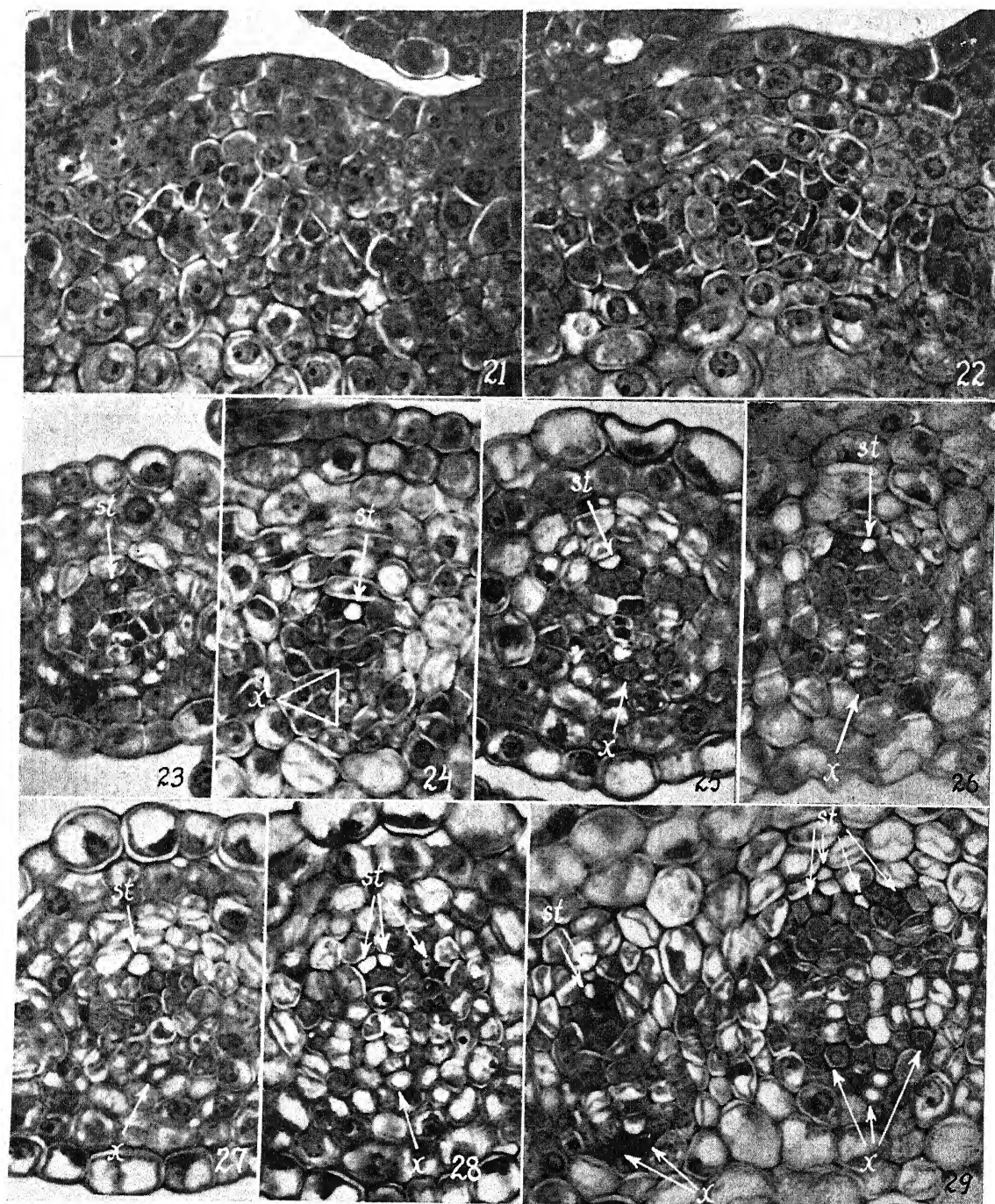


Fig. 21-29. Transverse sections of stem and leaves showing differentiation of procambium and of first vascular elements; *st*, sieve tube; *x*, xylem or xylem element. The stem sections in figures 21, 22, 24, 26, and 29 were taken the following number of microns below the apex, respectively: 90, 132, 306, 582, and 1,062. The sections in figures 23, 25, 27, and 28 were taken from leaves that were removed the following number of plastochrones from the apex, respectively: 36, 44, 45, 51. All figures $\times 750$.

off median (fig. 3), particularly if it is somewhat slanting, will reveal the continuity of the strand across the gap region.

In the present study the differentiation of the pro-

cambium in the flax axis has been followed in transverse and longitudinal sections from its inception to the early stages of phloem and xylem differentiation (fig. 21-29).

Using the tunica-corpus concept of the structure of apical meristems, the *Linum* apex is here classified as one having a single tunica layer overlying a corpus of about three layers of cells (fig. 5). Sometimes it appears as though there are two layers of tunica but periclinal divisions contributing cells toward the corpus are common in the second layer from the surface (fig. 5 at left above). The leaf primordia are initiated by periclinal and other divisions in the corpus (fig. 4, 13, and 15). Longitudinal divisions in the inner layer or layers of the corpus result in the formation of slightly elongated cells (fig. 13 and 15). These cells are the uppermost members of a strand of similar cells that may be followed downward into the axis to the level where these cells definitely assume the characteristics of the procambium (fig. 13 and 14, which show the same strand at two levels; fig. 4) and are connected with the procambium of other leaf traces. In the inner layers of the corpus periclinal divisions may be detected before such divisions in the outer corpus layer indicate the initiation of a leaf primordium. Figure 5, for example, shows at the left the repeated doubling up of the second corpus layer while the tunica and the first corpus layers remain single. The deep periclinal divisions in the flanks of the apical cone are concerned, however, not only with the formation of the procambium but also with the augmentation of the number of cortical layers. In similar manner, periclinal divisions in the outer corpus layer (second layer from the surface) are not restricted to the areas where leaves are initiated but occur also beneath the primordia (fig. 4 and 15). All these periclinal divisions occur in such close sequence that the products of the divisions tend to become aligned in oblique radial rows (note the cell rows indicated by arrows in fig. 4) in which it is difficult to distinguish between the future cortical and the future procambial cells until the degree of vacuolation in the two kinds of cells becomes conspicuously different (fig. 4), and the procambial cells assume their elongated narrow shape.

A summing up of the data on the longitudinal course of procambial differentiation in the flax apex gives the following sequence in the process. Periclinal and other longitudinal divisions proceed acropetally in the meristematic flanks of the axis tip as the latter becomes elevated through the activity of the apical meristem. Where leaf primordia do not arise, the subsurface products of the divisions become the cortical cells. Within the inner layers of the peripheral meristem divisions become particularly prominent beneath the areas where divisions in the outer corpus layer initiate the leaf primordia. These localized divisions, periclinal and others, form the procambial strands. The new procambial strands are continuous with the procambium of the older leaf traces. Thus the procambium of the shoot apex differentiates in a continuous acropetalous manner.

THE DIFFERENTIATION OF A PROCAMBIUM STRAND AS SEEN IN TRANSVERSE SECTIONS OF STEM AND LEAF.—As was already emphasized, a sharp demarcation between the procambium and the adjacent cells is

lacking in the early developmental stages of this meristem. Transverse sections clearly show that for a certain time the procambium strands increase in diameter not only by cell division within them but also by the addition of cells on their periphery from the surrounding tissue.

In figure 10 the two black arrows are pointing at small groups of cells that have resulted from recent divisions and by their position and density of protoplasts indicate that they are procambial cells. Figure 5 at level *e* (cells indicated by arrows) shows that similar recently divided cells in this region are elongated like young procambial cells. In figure 16 a procambial strand in earliest stages of development has been illustrated. (The procambial cells in this figure and also in figures 17 to 20 are marked with circles which represent nuclei.) The figure shows that some divisions have occurred—one is still in progress—around the small procambial strand. Some of the walls have been laid down parallel to the circumference of the strand, others in other planes. By these divisions cells are added to the periphery of the strand while divisions also continue within the strand itself. Since the divisions within the strand occur before the cells enlarge to the size of the mother cells from which they arose, the resulting procambial cells become conspicuously smaller in transverse section than the adjacent parenchyma cells. Figures 17 and 22 show procambial strands just before the beginning of phloem differentiation. To the right and left of these strands cells are dividing. New strands—the traces of higher leaves—will result from some of these divisions. A given transsection, therefore, contains procambial strands of different degree of differentiation depending on the age of the leaf with which they are connected.

While the divisions within the procambial strand continue and additional cells still become incorporated into them on their flanks the first vascular elements begin to differentiate. As is typical of the angiospermous shoots (see reviews by Esau, 1939, 1943), the first vascular cell to mature in a leaf trace is a photophloem sieve-tube element. The characteristic appearance and the sequence of developmental changes of the first sieve tubes in stems, roots, and leaves have been previously described by the writer (Esau, 1936, 1938, 1939, 1940). Therefore, the structure of these elements in flax is characterized only very briefly. The first sieve tubes may readily be distinguished in transverse sections by their somewhat thick primary walls which stain deeply with hematoxylin (fig. 24–29). The mature sieve-tube elements lack nuclei and their cytoplasm is scarce and stains very lightly, so that the cells appear as though they were free of contents. Immature protophloem sieve tubes have dense protoplasts (fig. 23; fig. 28, element at right arrow; fig. 29, elements lying to the extreme right and left in the group marked with arrows in the large bundle). The characteristic wall thickening appears before the nucleus disintegrates and the cytoplasm becomes thin (fig. 23, 28, and 29). These protophloem elements merit the des-

ignation as sieve-tube elements because they possess sieve plates.

The development of the procambium strands within the midvein of the leaf and within the trace follow a similar course as indicated by a comparison of figures 23, 25, 27, and 28, taken from leaves, with figures 17 to 20, 24, and 26, taken from stem sections.

Figures 18 and 23 show the appearance of a procambium strand during the late developmental stages of the first sieve-tube element. While this element matures (fig. 19 and 24) the procambial cells continue to divide longitudinally, particularly at the xylem pole. Most of these late procambial divisions occur in periclinal planes so that the new cells at the xylem end of the bundle become oriented in radial rows (fig. 19). Before many of these periclinal walls are produced a cell on the inner periphery of the procambial strand shows a conspicuous thickening and deep staining of the primary walls similar to those of the differentiating sieve tubes (cells marked *x* in fig. 20, 25, 26, and 29). These cells are the first xylem elements. In their subsequent stages of development the xylem elements develop lignified spiral secondary thickenings (fig. 27). In fully mature state they lack protoplasts (fig. 28; and 29, the two xylem cells indicated by the shortest arrow in the large bundle). The elements commonly have more or less oblique ends which overlap where two superposed elements are connected with each other. Whether the end walls are perforated has not been determined.

The tendency toward tangential orientation of the new walls produced by the later divisions of the cells which occur between the xylem and phloem poles becomes more pronounced with further bundle development. Moreover, the dividing cells become increasingly vacuolate (fig. 25-29). Eventually these cells are conspicuously set off from the densely cytoplasmic phloem and xylem regions as radially seriated vacuolated cells (fig. 29). Thus the procambium assumes certain characteristics of the cambium: high degree of vacuolation, predominance of tangential longitudinal divisions, and the resulting orderly radial seriation of the immediate products of division. The cells in the interfascicular areas keep pace with the radial increase in the size of the differentiating vascular bundles by occasional periclinal divisions (fig. 29). When at the end of primary growth of the axis the fascicular cambium becomes organized within the bundles, cambium appears also in the interfascicular areas, the periclinal divisions thereby becoming localized in a narrow zone, which is continuous with the fascicular cambial zone. As is typical of

seed plants (see Esau, 1938, p. 356-361; 1943), the transition from the primary to the secondary growth is gradual as seen in transverse sections of flax shoots.

SUMMARY

The procambium in the vegetative apices of *Linum perenne* L. is initiated in the form of strands, as a result of repeated longitudinal divisions within the peripheral meristematic region which becomes delimited as such because of the early differentiation of the pith.

The divisions initiating the procambium are localized beneath the areas where leaf primordia emerge and are evident during the earliest stages of leaf formation, at the time when the primordium does not yet form a bulge on the surface of the apical cone.

The leaf and its procambium are initiated immediately beneath the apical meristem. No distinct meristematic region is interpolated between the apical meristem and the procambium that would merit the designation of "meristem ring" or "prodesmogen" in the sense of Helm (1931) and Louis (1935), respectively.

The procambium strands differentiate in continuity with the procambium of the older traces within the axis and progress acropetally from the axis into the developing leaves. Thus the present study supports the emerging concept (Esau, 1943) that the procambium may differentiate within the axis as a continuous system, and not like the first xylem which appears as isolated segments that subsequently become connected.

In their early stages of differentiation the procambium strands are not clearly separated from the adjacent cells of the peripheral meristematic region. The morphologic differences between the procambium and the adjacent parenchyma develop gradually; furthermore for a time the procambium strands increase in size by the addition of cells on the periphery—cells resulting from divisions of adjacent nonprocambial cells.

Divisions occur also within the procambial strand and continue after the differentiation of the first vascular elements. In the procambium of a leaf trace a sieve-tube element is the first conducting cell to mature. Then the first xylem element matures. When the xylem begins to differentiate, the procambial divisions tend to become oriented tangentially.

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THE SIGNIFICANCE OF OXIDATION IN THE ENDODERMIS¹

D. S. Van Fleet

AS REPORTED earlier (Van Fleet, 1942) there is an oxidase system associated with the development of the endodermis in monocotyledonous and dicotyledonous plants. It is the belief of the author that this oxidase system is an active oxidation center responsible for the characteristic appearance and irregularity in the distribution of the endodermis in stem, root and leaf. In order better to understand the function and development of this tissue, experiments and observations were conducted which give a more exact picture of the nature of the oxidative processes and reveal some new facts about oxidation-reduction in the endodermis. A critical analysis of these facts makes quite clear the normal and characteristic interactions taking place between the endodermis and tissues of the stele and cortex.

Ecological and general habitat factors influencing the endodermis have been recognized by Schwendener (1883), Müller (1906), Haberlandt (1914), Van Fleet (1942) and others. Priestley and his students have demonstrated both internal and external environmental conditions believed to be connected with the functional development of the endodermis. It is hoped that the work reported here will help to clarify the relationship between external and internal factors recognized by earlier workers and will at the same time serve as a basis in method and approach to further work on this and other tissues which differentiate and function in terms of environmental factors.

METHODS OF STUDYING THE OXIDASE SYSTEM.—Freehand or sliding microtome sections of living roots and stems were made and immediately immersed in oxidase indicators. In a few cases the injection method was used to substantiate or verify the results of the application of indicators to fresh sec-

tions. The following indicators were used: guaiacol, para-phenylenediamine-hydrochloride + alpha naphthol, diphenylamine, benzidine, diphenylbenzidine, potassium-iodide-starch, titanium sulphate, etc. The indicator best suited for the endodermal oxidase system is guaiacol, because it does not oxidize in the absence of oxidase under alkaline conditions as do most of the above named indicators.

In order to modify the behavior of the oxidase system, plants were grown in various nutrient solutions that contained either pro-oxidants, antioxidants or inactive substances such as salts or organic substances. Much of the study of pro- and antioxidants was conducted on roots of *Allium* grown in solutions and in washed sand according to methods outlined in an earlier paper (Van Fleet, 1942). Solutions of pro-oxidants and antioxidants were also applied to fresh sections immediately before placing them in the indicators.

In addition to noting the behavior of the oxidase system, the distribution of the endodermis and the centripetal development and deposition of various substances were carefully observed in terms of the nutrient solutions used and in connection with oxidation-reduction processes taking place in the endodermis.

MECHANISM OF OXIDATION-REDUCTION SYSTEM IN THE ENDODERMIS.—By using various indicators, such as the Sudan stains, rosaniline, Scarlet R, iodine, potassium iodide or hydriodic acid, it is possible to demonstrate the presence of unsaturated fats and fatty acids in the plasma membrane and primary wall of the endodermal cell. These unsaturated substances of a suberogenic nature are deposited in the primary wall of the endodermis where they form peroxides at the unsaturated bond. Guaiacol, "Nadi" reagent, "Dopa" and other indicators are oxidized

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in the direct reaction, without introducing hydrogen peroxide, demonstrating that the peroxide is formed by an oxygenase system and further decomposed by peroxidases. The accumulation of decomposition products as a result of peroxide formation and decomposition may be demonstrated by applying orcinol, resorcinol, phloroglucinol and other similar indicators commonly used to demonstrate the presence of aldehydes. Other degradational products such as tannins and phlobaphenes accumulate at the same time that the aldehydes are formed. Extracts of tan-

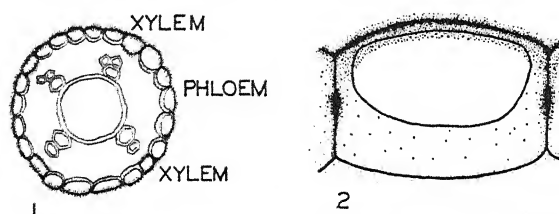


Fig. 1-2.—Fig. 1. Diagram to show oxidative action in the endodermis opposite the xylem and the phloem in a root. Oxidation, as indicated by stippling, is most intense in “gap cells” and in the outer tangential walls of the endodermis opposite the phloem.—Fig. 2. Diagram of a mature endodermal cell in which the most intensive oxidase activity is in the outer tangential wall. The oxidase action associated with the Casparian strip, a juvenile feature of the cell, is also indicated.

nins, from strips of tissue containing the endodermis, are easily precipitated by ferric chloride, iron alum, potassium ferrocyanide, etc., and these reagents give the characteristic color reactions indicating that tannins are present, and the application of dilute sulfuric acid frequently shows the bright red and characteristic color of phlobaphenes and other oxidation products derived from tannins. The common occurrence of phlobaphenes may be demonstrated by deep red-brown color in the presence of potassium hydroxide, by red color in acid, and by their easy solubility in methyl, ethyl, and propyl alcohol.

The unsaturated fats of the endodermis convert molecular to atomic or peroxide oxygen which brings about the oxidative decomposition of the fats and other accompanying substances, such as carbohydrates, tannins, etc., or indicators applied to the living system. This autoxidation of the unsaturated hydrocarbons in the endodermis may be made evident by the liberation of iodine from potassium iodide or dilute hydriodic acid with soluble starch as an indicator. The iodine liberated by oxidation of the unsaturated fats is immediately absorbed by the oxidizing system so that the plasma membrane and the walls of the endodermis become brown. The oxidation of various indicators (guaiacol, Nadi and Dopa) and the ability to absorb, point to the importance of the unsaturated substances of the endodermis as centers for the oxidation.

Priestly (1924) has proposed that unsaturated substances are oxidized as are the “drying oils” to become deposited as suberogenic materials. It has been demonstrated further that oxidation in the endodermis is like that of an oxidase system (Van Fleet, 1942); and, as has been pointed out here, the nature of the oxidase system is that of an unsaturated fat undergoing peroxide formation and decomposition. It is quite apparent that the unsaturated bonds converted to peroxide linkages become the energy source for further desaturation and peroxide promotion. The dehydrogenation of the fatty acid may be brought about by the formation and decomposition of the peroxides.

The question may arise as to whether the oxidase system of the endodermis may be called an oxidation system. Can it be demonstrated to be an oxidase system and at the same time be recognized as an active center for oxidation? The oxidation of the common indicators for oxidases is the basis for assuming the presence of an oxidase system. The autoxidation of any substance in such a way as to prevent the destruction of the system and at the same time to oxidize other foreign components or introduced indicators may be thought of as a type of oxidase system. According to Gallagher (1923) substances similar to

TABLE 1. Action of pro-oxidants on the oxidase system of the endodermis

Pro-oxidant	Concentr.	pH	Intensity of reaction	
			Endodermis	Other tissues
	%			
Cupric chloride	1	6-7	++++	++
Cupric sulphate	1	6-7	++++	++
Barium hydroxide	1-7	8-9	++	+
Sodium bicarbonate	1-3	8	+++	+++
Sodium borate	1	9	+++	+++
Sodium carbonate	1	9.5	+++	+++
Sodium oxalate	1	7	+++	+
Sodium perborate	1	8	+++++	+++
Sodium selenite	1	8.5	++++	+
Sodium selenite5	8	+++	+
Sodium selenite25	7.5	++	+
Sodium selenite12	7	+	++
Thiourea	1	6-7	++	+

lipins will bring about the oxidation of guaiacum in the presence of peroxidase. In the literature of industrial oils and fats there are many instances of spontaneous decomposition of unsaturated substances which behave as "oxidase systems," in that they oxidize the usual indicators, and at the same time actively oxidize the members of the system and other accompanying substances that may be present. Tauber (1940) and Strain (1941) report an unsaturated fat oxidase, present in legumes, which brings about peroxide formation in fats and as a result carotenoids present are oxidized.

PRO-OXIDANTS AND ANTIOXIDANTS.—The evidence obtained from a study of pro-oxidants and antioxidants substantiates the viewpoint that unsaturated fats are responsible for the oxidase reaction and that any substances inhibiting this reaction tend also to check the oxidation of fats and deposition of resultant degradational products in the endodermis. Those substances acting to accelerate oxidation through peroxidation are listed in table 1 as pro-oxidants. The action of pro-oxidants on the oxidase system of the endodermis is best studied by applying dilute solutions of the pro-oxidant to fresh living sections of stem or root followed by the application of some indicator such as Nadi reagent, guaiacol, diphenylbenzidine, etc. The plants best adapted for this study are young stems of *Asparagus*, *Smilax*, *Tradescantia* and *Vicia faba* and for root studies *Allium sativum*, and other species of onion or roots of peas, beans, or corn. The results of many trials of dilute solutions of inorganic and organic reagents are given in tables 1 and 2. The concentration and pH of the pro-oxidants

TABLE 2. Reagents acting as antioxidants

Acetone	Potassium sodium tartrate
Acetic acid	Potassium thiocyanate
Ammonium oxalate	Pyrocatechol
Ammonium sulphocyanide	Pyrogallol
Barium chloride	Pyroligneous acid
Carbonic acid	Sodium acetate
Chloral hydrate	Sodium ammonium phosphate
Cobalt chloride	Sodium benzoate
o-Cresol	Sodium chloride
Ferric chloride	Sodium citrate
Hydriodic acid	Sodium cyanide
Hydrogen sulfide	Sodium hyposulphite
Hydroquinone	Sodium nitrate
Iodine	Sodium nitrite
Potassium chloride	Sodium salicylate
Potassium ferricyanide	Sodium sulfate
Potassium ferrocyanide	Sodium sulfite
Potassium hydroxide	Sodium thiosulfate
Potassium iodide	o-Toluidine

are listed in each case along with the intensity of reaction that immediately followed their application. The intensity of peroxidation is recorded by taking the plus sign (+) as the reaction of the tissue without the application of either pro- or antioxidants.

It has long been recognized that the oxidation of an unsaturated fat is greatly increased under alkaline conditions, and the alkaline oxidation by hydro-

gen peroxide of a fatty acid, in the Dakin reaction, is a familiar one. The alkaline salts (table 1) function as pro-oxidants because they make the unsaturated fats more alkaline and bring about alkaline oxidation. The condensation of unsaturated substances and the accompanying oxidation are inhibited under acid conditions. Under stronger concentrations of alkaline salts the saponification of fats in the endodermal plasma and wall may be observed, and there is a partial saponification of fats taking place when the alkaline salts are present in dilute form. This partial saponification catalyzes peroxidation since free fatty acids are apparently conducive to the inception of oxidation. It is also possible that oxidation may accelerate saponification, at least this has been found to be true *in vitro* according to Stebnitz and Sommer (1937). The increased oxidase activity under alkaline conditions brings about a greater oxidation and deposition of unsaturated substances (Van Fleet, 1942), and the increase in oxidation under alkaline conditions is in keeping with the observations of Rose (1915) and Rose, Kraybill and Rose (1920).

It has been pointed out by Priestley (1924), and Lee and Priestley (1924), that fatty substances are saponified and migrate to surfaces as soaps and become deposited by evaporation and are then oxidized and undergo condensation. Further information in support of Priestley's belief has come from the work with pro-oxidants. The most effective pro-oxidants (table 1) are those that are capable of aiding in the saponification of fats in the endodermis (sodium selenite, sodium borate, etc.). This fact leads one to conclude that saponification in the plant may be accompanied by oxidation. Observations reveal that oxidation and saponification occur simultaneously at some surface where molecular oxygen is present. The endodermis as a bounding tissue at the inner edge of an aerating tissue, the cortex, becomes the locus for saponification and oxidation. The release of active oxygen that accompanies these reactions would of course catalyze saponification and at the same time it would bring about immediate deposition through condensation of the constituents of the system.

Sodium selenite (table 1) increases oxidase activity in the endodermis, but it decreases oxidase action in other tissues. Wright (1938) has demonstrated that selenite-poisoned mammalian tissues consume less oxygen and are not able to oxidize glucose but can oxidize p-phenylenediamine. With a decrease in concentration of selenite, oxidation in the endodermis diminishes and at the same time oxidase action in the cortex, hypodermis and epidermis is augmented. Sodium selenite augments oxidation in the endodermis at concentrations ranging from saturated solutions down to 1%, but below 1% the oxidation in the endodermis diminishes and in other tissues it increases. Under sodium selenite, oxidation in the endodermis is in direct proportion to the concentration, and at the same time oxidation in other tissues is in inverse ratio to the concentration of

selenite. Hence by using this reagent one may isolate histologically the oxidase system of the endodermis.

The alkaline salts decompose hydrogen peroxide compounds through their catalytic action and by their general alkaline condition which would render peroxides less stable. The alkaline pro-oxidants may conceivably be capable of increasing oxidation by decomposing peroxides and at the same time they saponify fats, which process is known to accelerate oxidative action. Saponification in the endodermal cell would lead to salt formation with resultant hydrolysis and ionization and the formation of alkali and free fatty acid. It is the belief of the author that salt formation and ionization accelerate oxidation.

Fresh alcoholic extracts or aqueous suspensions of chlorophyll act as pro-oxidants to the endodermal oxidase system and greatly increase the oxidation of indicators applied to the tissue after it has been covered with chlorophyll. Other surrounding tissues do not increase their oxidase activity. Chlorophyll acts as a catalyst both to saponification and oxidation through the action of the associated magnesium. Small amounts of magnesium chloride and copper chloride catalyze oxidation in the endodermis. Magnesium activates the oxidative decomposition of organic substances in the endodermis just as copper is already known to activate oxidation in commercial preparations of oils and gasoline.

It is not possible to reach any definite conclusion at present as to the part which copper plays in oxidation in the endodermis because copper will oxidize guaiacol (Moog, Garrigue, and Valdivié, 1939) and other indicators. Copper is apparently strongly adsorbed by the endodermal fatty system and acts as a part of the peroxidase reaction to release (O), and the Cu^{++} may be changed to Cu^+ by receiving electrons and hence oxidize indicators and other substances present.

Szent-Györgyi (1939) has pointed out that metal is alternately oxidized and reduced and thus becomes a catalyst through its change in valence. Under the influence of a catalytic metal, hydrogen atoms may be oxidized away from an organic substance to bring about dehydrogenation (autoxidation). Banga and Szent-Györgyi (1938) have demonstrated that Fe and Cu accelerate the autoxidation of dioximalcinic acid, and that here again electrons are passed in a chain reaction from metal atom to metal atom until finally the "electron acceptor," oxygen, is reached (Szent-Györgyi, 1939). Allott (1926) has shown that Fe increases the oxygen consumption of fats and fatty acids in the presence of glutathione. Smedley-Maclean and Pearce (1934) found that copper sulphate would bring about the *in vitro* oxidation of fatty acids in the presence of hydrogen peroxide. Ellis (1932, 1936) has studied the acceleration of autoxidation in fatty acids by catalysts. A peroxide is formed spontaneously according to Ellis, but it is possible to accelerate its formation by suitable catalysts (iron compounds), and Holtz (1936) has found that copper ascorbate and ferrous salts catalyze the *in vitro* oxidation of unsaturated fatty acids.

The organic antioxidants listed in table 2 inhibit oxidation in the endodermis just as they are also known to inhibit the autoxidation of fats (Olcott and Mattill, 1936). It is probable that they prevent the formation of peroxides in unsaturated substances associated with the endodermis. The inorganic antioxidants in table 2 are salts which are acid in aqueous solution and in this way they depress oxidase activity in the endodermis. Many of the organic and inorganic antioxidants have reducing properties, as for example, sodium sulfite, hydroquinone and pyrocatechol. Those which increase the acidity are ferric chloride, pyroligneous acid, sodium salicylate, etc. The following substances listed in table 2 have long been recognized as food preservatives and antioxidants: sulfur dioxide, sodium salicylate, boric acid, and benzoic acid.

Several dyes act as antioxidants in that they are absorbed by unsaturated substances which are then unable to undergo autoxidation. Rosaniline, Sudan III and IV, and Scarlet R act as excellent indicators for unsaturated fatty acids which are then prevented from acting as oxidase centers. This fact suggests that many of the antioxidants may act to prevent unsaturated bonds from forming peroxides and undergoing further oxidative decomposition.

The addition of tannic extracts from the bulb scales of *Allium* to nutrient media, in which roots were growing, greatly decreased suberization and condensation in the endodermis (Van Fleet, 1942). Tannins added to fresh sections prevented oxidation. It is probable that, as proposed by Moureau and Dufraisse (1922), the addition of tannins to autoxidizable compounds prevents the formation of peroxides. Tannins may then be thought of as antioxidants. The presence of antioxidants in aerial stems is the probable cause for the failure of the endodermis to put in an appearance, and it may well be that tannins produced during periods of active growth are responsible for inhibition of oxidation at the locus of the endodermis.

THE SIGNIFICANCE OF THE OXIDATION-REDUCTION SYSTEM.—Oxidation in the endodermis is initiated by the unsaturated fats and fatty acids characteristic of this tissue. Oxidation proceeds in the step-wise fashion associated with oxidase systems (diagram 1), and these oxidative reactions result in the molecular degradation of unsaturated and associated substances which are deposited in the endodermal wall. The importance of free molecular oxygen to this reaction has been demonstrated (Van Fleet, 1942). It is quite evident that the oxidation by peroxide or nascent oxygen must be preceded by two distinct steps, (1) the oxygenation of the oxygen acceptor, and (2) the action of peroxidase to split and release the peroxide oxygen from the acceptor. The accumulation and condensation of aldehydes, lignic and suberic substances follows the active period of autoxidation. The unsaturated fatty acids which are the centers for the oxidase system are oxidized to aldehydes and other decomposition products, and at the same time the energy thus released may result

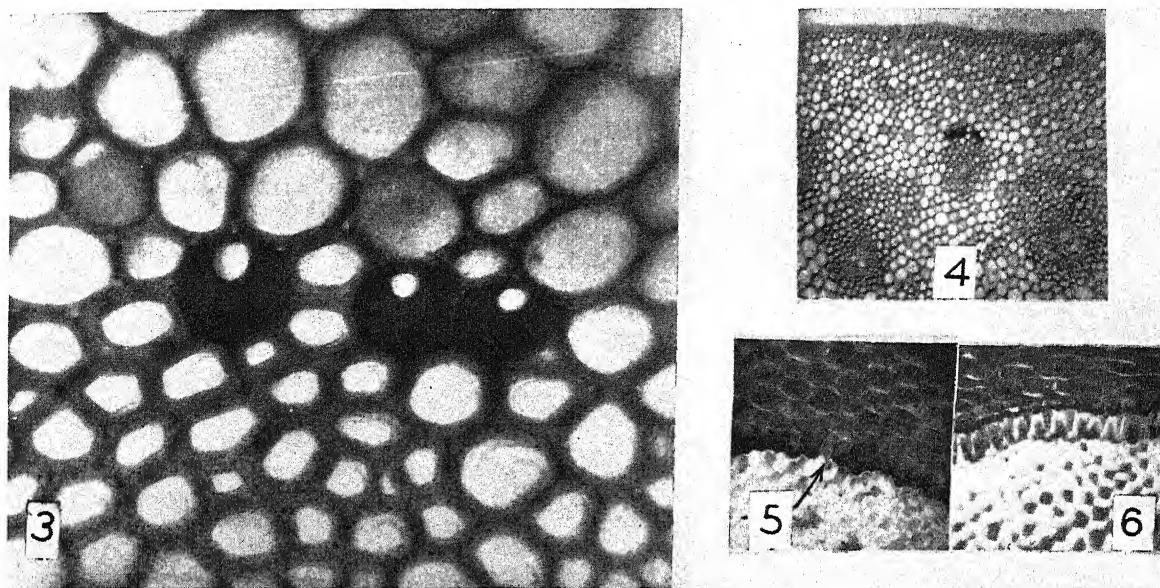


Fig. 3-6.—Fig. 3. Photomicrograph of *Smilax rotundifolia* stem section, from the region of the ground line, to show a mature interrupted endodermis. Cells are dark because of an accumulation of oxidation and degradational substances. $\times 520$.—Fig. 4. Same as figure 3, showing location of endodermis on peripheral side of bundle. $\times 86$.—Fig. 5. Photomicrograph, by polarized light, of *Smilax* root, showing deposited oxidation products and "gap cell" at end of arrow. $\times 86$.—Fig. 6. Photomicrograph, by polarized light, showing isotropic zone of oxidation in the outer tangential wall of the endodermis and in the cortical layer outside the endodermis.

in the formation of unsaturated substances from the aldehydes. Release of (O) activates condensation reactions, and aldehydes, hydroxy-acids and fatty acids are polymerized. The addition of dilute

(0.0003%) quantities of hydrogen peroxide to sections and the resultant "browning" and condensation of tannins illustrates this point.

The mature endodermis is heavily supplied with oxidized aromatics and generic lignic materials in a basic matrix of cellulose. These polymerized products may accumulate to the point where they act as antioxidants and halt oxidation. The cells in figures 3, 4 and 5 gave only a weak oxidase test. In some *Smilax* roots there is a secondary zone of oxidation and deposition in the outer tangential wall of the endodermis and in the first cortical layer of cells outside the endodermis. This secondary oxidation zone is isotropic in polarized light as shown in figure 6. This oxidation zone develops after the first zone of oxidation in the endodermis has declined in oxidative deposition. As shown in figure 8 there is also an accumulation of condensation substances inside the endodermis in the stele.

The centripetal nature of deposition in the endodermis as it relates to the oxidation-reduction system.—The maturing endodermis is characteristically thickened to a much greater extent on the radial and inner tangential walls to give a centripetal type of deposition (fig. 3). The most intensive oxidase activity is, as shown in figure 2, in the immediate region of the outer tangential wall. The products of this oxidation accumulate in the inner part of the cell along with cellulose lamellae. In young endodermal cells opposite the phloem the oxidase activity is always most intense in the outer tangential portions, but in the endodermis opposite the xylem, oxidases

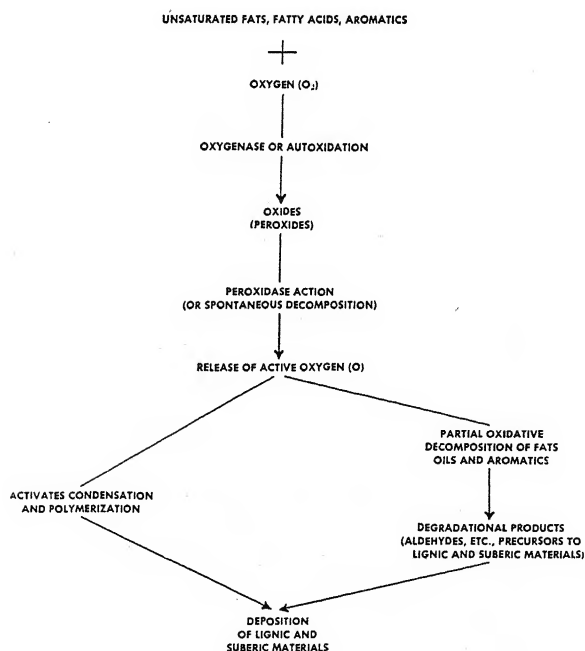


Diagram 1.—Significance and general trend of oxidation in the endodermis.

are distributed around the entire protoplast and around all walls. The more intense oxidase activity in the outer portion of the endodermis apparently prevents deposition in that part of the cell but brings about deposition in the inner portion by giving rise to the condensation substances. The appearance of the endodermis as a series of U-shaped cells is thus related to the intense oxidation in the outer tangential wall plus the concomitant condensation and polymerization that take place along the radial walls and the inner tangential wall. The lack of true cellulose in the outer peripheral part of the U-shaped endodermis is a result of the intense oxidation of unsaturated substances. The oxidation of unsaturated fats may bring about the oxidation and destruction of other substances, notably cellulose or cellulose precursors. The typical endodermal cell as seen in many monocotyledonous plants may be thought of as being composed of an outer or peripheral surface undergoing oxidation and dehydration while deeper portions of the cell and inner surfaces bring about the condensation of products resulting from oxidase activity.

The failure of inner portions of the young and maturing endodermal cell to oxidize indicators, tannins and chromogens present in the endodermis suggests that the inner region of deposition is either a zone of reduction or a "neutral" zone. One may then arrive at the logical conclusion that the greater prevalence of unsaturated fats in the outer tangential wall (Sudan stains, Scarlet R, chlorophyll, Rosaniline, etc.) is directly correlated with more intense oxidative action in this region, and it becomes evident that the endodermal cell (with the exception of "gap" cells opposite the xylem) is composed of an outer zone of higher oxidation potential and an inner zone which is at least low in oxidative properties if not a zone of reduction.

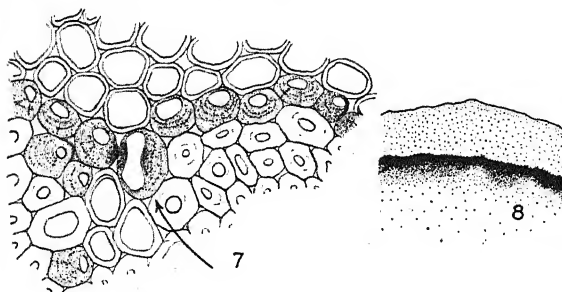


Fig. 7-8.—Fig. 7. Camera lucida drawing of mature endodermis in *Smilax rotundifolia* stem to show the occurrence of thin walls in the endodermis adjacent to thin-walled cortical or sclericycle cells. Arrow points to asymmetrical cell associated with thinly lamellated cells in both cortex and stele. $\times 520$.—Fig. 8. Diagram of *Smilax* stem to show accumulation of oxidized and polymerized substances in the endodermis and in the stele inside of the endodermis. $\times 70$.

In the inner or stelar side of the endodermis there is an accumulation of unsaturated materials and a concomitant but slight oxidation. There is a condensation of carbohydrates and fats to form cellulose

on the one hand and small amounts of suberic substances on the other. The failure of these materials to remain in an "active" condition is probably brought about by the influence of depositional factors in the underlying sclericycle and stelar elements. There is an accumulation of oxidized and polymerized substances in the lamellae of the endodermis nearest the stele (fig. 7) and also a slow deposition of similar material in underlying fibers during the growing season (fig. 8).

The unilateral deposition of cellulose lamellae to give the typical asymmetrical endodermal cell (fig. 3 and 7), so often seen in monocotyledons, points to an additional fact about the oxidation-reduction system. According to Hilditch (1940) it is possible for a carbohydrate system to be converted by reduction into a fatty system by an oxidation-reduction process in which hydrogen peroxide or atomic oxygen would be released and utilized by some substance, or oxygen acceptor, present in the system. The thin cellulose lamellae on the cortical side of the endodermis may be indicative of the conversion of carbohydrates to fats on this side of the endodermis. The abundant presence of unsaturated fats and peroxides on the cortical side of the endodermis, and the almost complete absence of cellulose in this zone would seem to be evidence of an oxidation-reduction conversion of carbohydrates such as Hilditch has suggested.

Relationship of endodermal oxidation to the xylem, phloem and cortex.—Fatty precursors to oxidase activity may originate in the phloem (Van Fleet, 1942), at least fatty oxidation and deposition first take place opposite the phloem. Unsaturated fats and oxidation may be noted later in the endodermis opposite the xylem. After oxidase activity opposite the xylem is initiated, it remains more intensive than opposite the phloem. Oxidase activity in cells opposite the xylem is not confined to the outer tangential wall as in the case of phloem but is found in and around all walls (fig. 1). The sustained oxidase activity in all parts of the endodermis opposite the xylem is the probable reason for the lack of centripetal deposition in these cells resulting in the typical "gap cell" so often associated with the xylem (fig. 1 and 5). Oxidative action is in general apparently stronger in the outer tangential wall and outer portions of the endodermis opposite the phloem than it is in any part of the endodermal cell opposite the xylem. This more intensive oxidation would of course lead to greater deposition and condensation opposite the phloem.

The greatest deposition in the endodermis of cellulose, lignic and suberic substances is away from the thin-walled cortical cells and is toward or next to the thick-walled and lignified sclericycle (fig. 3 and 7). This occurrence of the thinnest wall of the endodermis next to thin-walled cortical cells is strikingly shown in the asymmetrical cells one often encounters in *Smilax* stems. In figure 7 one of the endodermal cells has a dumb-bell-shaped lumen as a result of the concomitant occurrence of thin-walled parenchymal cells on both sides of the endodermis.

There are two thinly laminated areas in this asymmetrical cell, one opposite a cortical cell and the other nearest to a stelar cell, both of which are relatively thin-walled and are characterized by intense oxidative activity.

Oxidation is, as indicated earlier in this paper, more intense in the outer portion of the endodermis toward the periphery (fig. 6), and it is also true that oxidation is in general more pronounced in the cortex than it is in the stele or sclericycle. The thin-walled parenchymal cells infrequently found in the sclericycle (fig. 8) may also show more oxidative action. From the above observed facts, it would seem to follow that unilateral deposition is related to differences in intensity in oxidation and deposition. The endodermis as a differential between two widely different systems, the cortex and the stele, thus exhibits the characteristics of both systems, that is, the endodermis is more like the thin-walled cortical cells on one side and the thick-walled sclericycle cells on the other. Thus unilateral deposition of U-shaped cells is indicative of a differential between a zone of deposition and one of extremes in oxidation-reduction.

The cortical side of the endodermis is next to a fairly constant oxygen supply which would allow for immediate absorption of oxygen and, following this, peroxidation and resultant oxidation. The polymerization and condensation of oxidized products would necessarily follow at a slower rate and there is evidence, as reported earlier, that condensation and deposition of oxidation products proceed according to the amount of water in the cortex and the environmental medium.

The significance of the endodermis as a center for oxidation and as a differential between two extremely different tissue systems is closely linked with its function as a mature tissue. The mature endodermis with greatly thickened walls stops the outward diffusion of fatty substances from the stele. A plant with a functional endodermis shows only a slight oxidase activity in the cortex, and if the endodermis is interrupted or broken there are corresponding sections in the cortex where oxidation is much more pronounced (Van Fleet, 1942). The endodermis thus becomes a barrier to a part or all of the constituents of oxidase systems.

The distribution and differentiation of the endodermis as a result of oxidation processes.—The complete absence of the endodermis in aerial parts of most monocotyledonous plants (Müller, 1906, and Van Fleet, 1942) and the infrequent appearance of this tissue in above-ground parts of many dicotyledonous plants (Priestley and students) has led to much conjecture concerning the factors responsible for its appearance. According to Priestley (1926) the fatty substances in the stem in darkness do not move to the surface but are oxidized and deposited in the endodermis as a Casparian strip. In the stem in the light, fatty substances migrate immediately to the stem surface where they form the cuticle. At the inner walls of the endodermis fatty substances col-

lect and are oxidized because they come in contact with air from the large intercellular spaces of the cortex. Thus Priestley believes that in aerial stems the cortical oxygen supply is less than in roots and underground stems, hence the fats are not condensed but migrate to the epidermis to become a part of the cuticle. Priestley and his students also believe that there is less water loss in aerial stems where the cuticle is thick, hence condensation does not take place in the site of the future endodermis.

The behavior of the oxidation system of the endodermis is causally related to the appearance of this tissue; however, it is not possible as yet to propose a complete theory as to the function of the oxidation system in bringing about the formation of a differential tissue between stele and cortex. The following are proposed as theories to explain the absence or irregular appearance of the endodermis in aerial stems. (1) Autoxidation is catalyzed and carried to completion without the deposition of intermediate products. (2) Some photocatalyst may be present which breaks up or accelerates oxidation by catalyzing the reaction of some constituent of the oxidation system. (3) There may be less peroxide formed in aerial stems in the period of active growth, or peroxides may be immediately decomposed so as to prevent the oxidation and condensation of unsaturated substances. (4) Antioxidants may be present in greater quantity or in a more active form in aerial parts of the plant. (5) Inhibitors in the form of antioxidants may be present in both aerial and subaerial stems but they may affect the light reaction and not the oxidation reaction in the dark. (6) The presence of antioxidants in vegetable oils and fats (Bradway and Mattill, 1934, and Olcott and Mattill, 1936) prevents autoxidation, and it is conceivable that there may be more antioxidants formed in the light than in the dark. (7) The distribution of unsaturated fats, or the nature of these fats, may affect the differentiation of the endodermis.

The fact that many monocotyledonous plants lack an endodermis in both subaerial and aerial stems, and that still others have an endodermis in aerial stems (Van Fleet, 1942), suggests that there must be some exact oxidation system responsible for endodermal differentiation. The fact that the Araceae in general lack an endodermis in stems regardless of their environmental position suggests an internal agency responsible for differentiation. In the Gramineae (Schwendener, 1890) the irregular and sporadic appearance of the "mestome sheath," an endodermal tissue, its absence in whole tribes and presence in others, is further suggestive of something more than the quantitative cortical oxidation and condensation of fats. If light and other external environmental factors were alone responsible for fat mobility then there should be uniformity in endodermal formation. The irregular appearance of the endodermis must be related to either (1) some variation in behavior of constituents, or (2) the irregular distribution of inhibitors to the oxidation reaction.

SUMMARY

Evidence obtained by the use of various indicators, numerous oxidants and antioxidants, substantiates the viewpoint that unsaturated fats associated with the endodermis autoxidize and undergo peroxidation to release an active form of oxygen. The peroxide formation by fats is construed as an oxidase system resulting in the oxidation and reduction of accompanying substances as well as the components of the system. Autoxidation is most rapid under alkaline conditions and in the absence of tannins, reducing agents and other inhibitors. Unsaturated bonds converted to peroxide linkages are believed to be the energy source for further desaturation and peroxidation. The release of active oxygen and the presence of unsaturated substances accelerate condensation reactions, and a polymerization of degradational products leads to the formation of a typical mature endodermis characterized by the Casparian strip or centripetal deposition in the walls.

The typical endodermis as a series of U-shaped cells, particularly in endogenous plants, is a result of intense oxidation in the outer tangential walls and concomitant condensation and polymerization along the radial and inner tangential walls. The greater prevalence of unsaturated substances in the outer tangential walls is directly correlated with more intense oxidative action in this region. The lack of thick cellulose lamellae in the outer part of the U-shaped endodermis is thought to be a result of the intense oxidation in this zone. The oxidation of unsaturated fats is believed to bring about the oxidation and destruction of other substances, notably cellulose or cellulose precursors. The endodermal cell may be thought of as a differential tissue in which the outer side has a high oxidation potential, and the inner zone is low in oxidative properties and possibly constitutes a zone of reduction.

Fatty deposition and oxidation in the endodermis are initiated opposite the phloem which accounts for

the early deposition of centripetal lamellae in the region of the phloem. Oxidative action comes later in the endodermis opposite the xylem than in the region of the phloem, and oxidation in some cells opposite the xylem is not confined to the outer tangential wall as in the case of the phloem but is found in and around all walls. The sustained oxidase activity in all walls of the endodermis opposite the xylem is the probable reason for the lack of centripetal deposition in these cells, thus giving rise to the typical "gap cell" so often associated with the xylem.

The endodermis as a differential between two widely different systems, the cortex and the stele, exhibits the characteristics of both systems, that is, the endodermis is more like the thin-walled cortical cells on one side and the thick-walled sclericycle cells on the other. Oxidation is more pronounced in the outer portion of the endodermis toward the cortex and is in general less intense in the stele or sclericycle. Unilateral deposition is related to differences in intensity of oxidation and deposition, and the U-shaped cells are thus indicative of the endodermis as a differential between two very different tissue systems.

The irregular distribution of the endodermis is believed to result from the behavior of the oxidation system herein proposed, and several theories are offered as to the function of the oxidation system in the formation of this tissue. The interrupted nature of the endodermis, its absence in aerial stems of many monocotyledonous plants and irregular appearance in dicotyledonous plants, are believed to result either from some variation in behavior of constituents of the oxidation system, or from the irregular distribution of inhibitors to the oxidation reaction.

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THE EFFECTS OF AGE AND IRRADIATION ON CHROMOSOMAL ABERRATIONS IN ALLIUM SEED ¹

Charles Nichols, Jr.

ANALYSIS of chromosomal aberrations has become an important part of experimental cytology. Chromosomal rearrangements have been shown to be of considerable importance in relation to speciation (Dobzhansky, 1937). These changes are known to occur spontaneously in many organisms, but their frequency is very low, and for this reason they are difficult to study quantitatively. Aging, heat, irradiation, and hybridity bring about an increase in the frequency of structural chromosome alterations as well as an increase in the frequency of gene mutations. How these factors are related to one another and how they cause changes in the chromosome and gene mutation rates are not well understood. However, the fact that all these external agents cause similar changes suggests that a broad fundamental process is the primary cause of increases in mutation rates.

The present investigation deals with gross structural changes in the chromosomes of *Allium* induced by subjecting the dormant seed to the action of age and irradiation. Although the effects of these external factors have been studied by many investigators dealing with both plant and animal material, there are very few reliable data concerning the seed.

Cartledge and Blakeslee (1934) studied the effects of aging on *Datura* seed and Navaschin (1933) and Navaschin and Gerassimowa (1936) reported on the effects of aging on *Crepis* seed. Peto (1933) studied the effects of high temperature on the mutation rate in corn and barley seed. Schkwarnikow and Navaschin (1934) and Kirnossowa (1936) studied the effects of high temperature and moisture content on seeds of *Crepis*. However, none of these investigators give quantitative data on the number

of chromosome aberrations appearing immediately after germination. Gustafsson (1937) studied the effects of age, moisture, and X-rays on the seed of barley, and reports on the number of disturbed cells in the first mitotic divisions after germination. Nichols (1941) has reported on spontaneous aberrations and the effects of aging on *Allium* seed as determined from examination of the first mitotic divisions.

Dry seed have many advantages as material for experimental studies on chromosome behavior. Seeds are in a relatively dehydrated condition and physiological activity is at a minimum. Seeds of many plants can be kept viable for a number of years and thus the effects of aging may be investigated. Seeds are also more resistant to external agents such as heat and X-rays. This enables a wide range of treatments to be used when dealing with these external factors. Finally, the chromosomes are known to be in the resting stage in dormant seed, and thus any treatment applied to the seed must act on the chromosomes while in a relatively uniform condition.

In the experiments to be reported here seed of several different commercial varieties of *Allium cepa* L. were used. The seeds were obtained from a reputable seed company and represent crops from the years 1935, 1936, 1937, 1938, 1939, and 1940. All seeds were germinated at room temperature on moist filter paper in Petri dishes. Germination took place in three to four days depending on the condition and the treatment of the seed. Cells in the very shortest root tips contain no division figures. For this reason it seems probable that the first visible signs of germination in *Allium* are the result of cell expansion. An examination of root tips of different lengths indicated that the first mitotic activity was to be found in root tips 2-4 mm. in length.

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Primary mitotic divisions may be distinguished from secondary and later divisions by the nature of the aberrations observed. In the primary divisions chromosome bridges are accompanied by acentric fragments. The fragments in primary divisions are very definite in shape and outline, while in secondary divisions they appear as micronuclei in various stages of disintegration. Bridges and fragments occurring during the last division preceding dormancy would appear as secondaries in the first division after dormancy. However, the frequency of such aberrations in the last division before dormancy is probably very low. The few cells observed which contained bridges without fragments are probably due to the fact that the fragments were too small to be seen.

TABLE 1. *Distribution of the various types of aberrations observed.*

Types of aberrations	Number	Per cent
Chromatid aberrations:		
Bridge plus fragment	44	1.4
Bridge without fragment	18	0.5
Acentric rod fragment	100	3.2
Acentric dot fragment	12	0.3
Chromosome aberrations:		
Bridge plus fragments	781	25.0
Bridge without fragments	218	7.0
Paired acentric rod fragments.....	1135	36.4
Paired acentric dot fragments.....	801	25.7
Ring chromosomes	4	0.1
	3113	99.6

All root tips were fixed in a 3:1 alcohol-acetic fixative and stained according to the Feulgen technique. Smear preparations were used entirely and anaphase figures were analyzed in most cases, since chromosome bridges and fragments are most readily detected at this stage. Wherever possible 100 figures were analyzed on each of three slides. A total of 21,808 cells was tabulated and 3,113 aberrant cells were recorded. The distribution of the various types of aberrations is given in table 1.

EFFECTS OF IRRADIATION AND AGE ON DRY SEED.—Ten lots of dry seed consisting of five different varieties and two age classes in each variety were selected for irradiation. The dry seeds were given a dose of 2000r at an intensity of 100r/minute. The irradiated seeds were divided into three equal parts. One part was placed in Petri dishes to germinate immediately after treatment and roots 2–4 mm. in length were fixed. In all cases the irradiation resulted in a significant increase in the frequency of gross chromosomal aberrations. The results of this experiment are presented in table 2. The object of this analysis was to determine whether there were any varietal differences in sensitivity to X-rays and whether the age of the seed had any effect on sensitivity. Four of the five varieties show about the same sensitivity to X-rays, but the variety Sweet Spanish appears to be somewhat less sensitive. This fact is of interest because this variety shows a higher frequency of aberrations in the controls than do the other four varieties.

In order to determine whether sensitivity to X-rays is in any way related to the age of the seed the correlation between age and X-ray sensitivity was calculated for the ten lots of seed tested. A coefficient of +.12 indicates that there is no significant correlation between these two factors and suggests that, while both age and irradiation cause increases in the frequency of chromosomal aberrations, the causal mechanisms may be different.

Of the remaining two lots of seed which were irradiated, one part was allowed to remain dry at room temperature for two weeks before germinating, and the third part was kept dry at room temperature for four weeks before being placed in Petri dishes to germinate. In this way it was possible to determine whether there were any secondary after-effects of irradiation such as have been reported by Gustafsson (1937). The results are presented in table 3. It is quite evident that there is a definite after-effect following X-ray treatment of dry seed which causes a marked increase in the frequency of chromosomal disturbances. It is further evident that in certain cases this after-effect is quite rapid, since the great-

TABLE 2. *A comparison of controls and irradiated seed in several varieties and age classes of Allium.*

Variety and age	Total cells analyzed	Controls Per cent aberrant cells	Total cells analyzed	2000r Per cent aberrant cells	Difference
Yellow Strassburg 1935	330	10.4	261	19.4	9.0 ± 2.8
Yellow Strassburg 1936	319	8.4	323	13.8	5.4 ± 2.2
Mammoth Silver King 1936....	349	5.8	314	11.0	5.2 ± 2.0
Mammoth Silver King 1937....	302	5.6	324	13.3	7.7 ± 2.2
Ohio Yellow Globe 1936.....	311	8.9	315	16.8	7.9 ± 2.5
Ohio Yellow Globe 1937.....	327	3.9	344	11.3	7.4 ± 2.0
Sweet Spanish 1936	216	15.0	234	20.0	5.0 ± 3.5
Sweet Spanish 1939	315	5.7	235	9.8	4.1 ± 2.1
Ebenazar 1938	305	2.6	222	13.0	10.4 ± 2.4
Ebenazar 1940	227	0.8	291	5.8	5.0 ± 1.4

TABLE 3. *Summary of the effects of delayed germination following irradiation.*

Variety and age	Examined immediately		After two weeks		After four weeks	
	Total cells Analyzed	Per cent aberrant cells	Total cells analyzed	Per cent aberrant cells	Total cells analyzed	Per cent aberrant cells
Yellow Strassburg 1935...	261	19.4	303	31.9	303	30.4
Yellow Strassburg 1936...	323	13.8	227	27.2	312	26.3
Mammoth Silver King 1936	314	11.0	235	14.8	314	21.6
Mammoth Silver King 1937	324	13.3	306	16.9	306	21.7
Ohio Yellow Globe 1936...	315	16.8	220	22.0	300	29.0
Ohio Yellow Globe 1937...	344	11.3	321	21.7	343	20.4
Sweet Spanish 1936.....	234	20.0	184	37.5	255	32.1
Sweet Spanish 1939	235	9.8	190	13.6	210	25.5
Ebenazar 1938	222	13.0	212	25.4
Ebenazar 1940	291	5.8	316	2.8
Mean per cent aberrant cells for all material examined immediately after irradiation:						
13.24 \pm .633.						
Mean per cent aberrant cells for all material examined two weeks after irradiation:						
23.30 \pm .943.						
Mean per cent aberrant cells for all material examined four weeks after irradiation:						
23.53 \pm .791.						

est increase in frequency of aberrations takes place in the first two weeks following irradiation, and little change is observed during the third and fourth weeks. Only one out of the ten lots of seed thus tested failed to show after-effects. This one was the only lot of fresh seed used, Ebenazar 1940.

A second series of tests was carried out to determine whether the increase in aberrations with delayed germination following X-ray treatment was in any way associated with the age of the seed. For this purpose one lot of five-year old seed and two lots of fresh seed were used. Following an X-ray dose of 2000r, part of the seeds in each lot were germinated immediately, and the remaining seeds were germinated at five-day intervals for a period of three weeks. The results are given in table 4.

It is apparent from these results that in aged seed, in addition to aberrations caused by any direct-hit mechanism of the X-rays, there are set in motion other changes in the cells which continue to cause chromosome breakage for some time after irradiation has ceased. The fact that fresh seed does not show this after-effect would indicate that the physiological condition of the cells is intimately associated with the production of after-effects following irradiation with X-rays.

By varying the treatment of the seed following irradiation it is possible to obtain further evidence that the physiological condition of the seed is closely

associated with the cytological after-effects resulting from delayed germination. A single lot of seed was given an X-ray dose of 2000r. Immediately following irradiation the seeds were divided into six equal parts and treated as follows: (1) germinated immediately, (2) kept seventy-two hours dry in the refrigerator, (3) kept seventy-two hours moist in the refrigerator, (4) kept seventy-two hours dry at 60°C., (5) kept seventy-two hours dry at room temperature, (6) kept 144 hours dry at room temperature. This experiment was carried out in duplicate, and the results are summarized in table 5.

The seeds kept moist at the low temperature show a significant increase in the number of aberrant cells. This is presumably not an effect of the low temperature, since the seeds kept dry at that temperature did not show any increase. It may be due entirely to the moisture or to an interaction of moisture and low temperature.

Discussion.—The results of the preceding experiments pertaining to the frequencies of chromosomal aberrations in dormant seed may shed some light on several points of theoretical interest to the experimental cytologist. It is a generally accepted fact that the actual frequency of breaks in X-rayed material is very much greater than can be determined by analyzing chromosome aberrations. This is due to the fact that in the majority of cases single breaks in a chromosome are followed by a re-fusion of the

TABLE 4. *Comparison of the effects of delayed germination, as shown by per cent aberrant cells, following irradiation of one lot of aged seed and two lots of fresh seed.*

Variety and age	Rest period following irradiation, in days				
	0	5	10	15	20
Yellow Strassburg 1935	17.0	21.4	30.1	23.0	23.1
Prizetaker 1940	8.7	7.7	8.7	8.2	6.2
Red Wethersfield 1940	9.9	10.6	8.7	9.0	8.0

TABLE 5. Effects of varying the conditions of storage during the period of delayed germination.

Treatment	Total cells analyzed	Per cent aberrant cells
2000r germinated immediately	658	8.9
	378	9.2
2000r kept 72 hrs. dry in refrigerator . .	503	8.4

2000r kept 72 hrs. moist in refrigerator	459	17.0
	312	18.2
2000r kept 72 hrs. dry at 60°C.	415	10.3
	339	8.5
2000r kept 72 hrs. at room temperature	338	10.9
	377	12.4
2000r kept 144 hrs. at room temperature	509	14.3
	352	12.4

broken ends in the original position, leaving no evidence of breakage. Sax (1939) has discussed this point in a paper dealing with the time factor in the production of chromosome aberrations by X-rays.

In dealing with chromosome aberrations due to aging, the exact mechanism causing chromosome breakage is not known, but it is apparent that one cannot speak of "one hit" and "two hit" aberrations in the same sense that these terms are used in connection with X-ray-induced aberrations. Whatever the mechanism may be, it is similar to irradiation in that it probably produces a great many more breaks than can be detected by an analysis of chromosome aberrations at anaphase. Of interest in this connection is the question as to how long broken ends of chromosomes can remain open. In *Tradescantia* microspores Sax (1939) finds that broken ends remain in an unstable condition for not more than one hour before healing. Stadler (1932), on the other hand, believes that the broken ends of acentric fragments remain unhealed and capable of re-fusion for several cell generations. Recent evidence from work on X-ray-induced structural changes in *Drosophila* (Muller, 1940; Kaufmann, 1941) indicates that broken ends in sperm nuclei remain unhealed for as long as a month. Muller reports that the primary effects of irradiation (breakages) accumulate in the spermatozoa until the time of fertilization, regardless of how long this period may be.

In connection with aberrations arising as a result of aging or other treatments of dry seed, three hypotheses are possible: first, breakage and re-fusion may be occurring at random during the period of dormancy; second, breakage may be occurring at random, but re-fusion may not take place until the initiation of activity at the time of germination; third, breakage and re-fusion may not occur until the time at which germination starts.

If broken ends of chromosomes in the resting nuclei of dry seed of *Allium* remain unstable and capable of re-fusion for only a short time, then the actual frequency of breakage in these chromosomes must be very high in order that two independent

breaks occur within the given time limit and thus give rise to reunions which result in visible aberrations. It would seem more likely that the broken ends of chromosomes in dormant seed can remain in an unstable condition for long periods of time. Although certain difficulties in the material have prevented an adequate test of the time factor in the production of X-ray-induced aberrations in *Allium* seed, it is not inconceivable that the broken ends may remain open throughout the period of dormancy of the seed. All the reunions may occur at the time when germination is initiated.

It has been suggested that aberrations observed in aged seed might be due to the accumulation of the effects of natural radiation, but Navaschin (1933) and others have shown that this cannot be the case. The results of many investigators on the relationship of X-ray dosage to percentage of structural changes has led to the belief by some that a direct-hit mechanism is involved. However, the fact that agents other than X-rays can produce the same effects leads one to question the validity of the direct-hit mechanism as of general application. It is probable that X-rays produce two independent changes: a direct-hit mechanism causing chromosome breakage and fusion, and a physiological effect. In fresh seed the physiological effect does not alter the frequency of chromosome breaks. In aged seed, however, the physiological effect results in an increase in the frequency of chromosome breaks. Breaks resulting from the physiological effects of the X-rays are superimposed on the breaks resulting from the direct-hit mechanism. Changes in the coiling cycle causing stresses on the chromosomes can hardly be involved in the dry seed, since the chromosomes are in the resting stage and in a relatively relaxed condition. It is possible that the frequency of chromosomal aberrations is to a certain extent genetically controlled. Beadle (1937) has shown that the gene for sticky chromosomes in maize causes an increase in the frequency of chromosomal rearrangements. The most plausible explanation seems to be that internal changes in the physiological condition of the cells are responsible for chromosome breakage (Schkwarnikow and Navaschin, 1934; Stubbe, 1936).

In connection with changes in the physiological condition of the cells, there is some disagreement as to whether direct changes in the chromosomes cause the increase in break frequency (Gustafsson, 1937) or whether the chromosomes are indirectly affected as a result of a general upset in the metabolism of the cells (Schkwarnikow and Navaschin, 1934). At present, experimental proof of either hypothesis is lacking. The investigations of *Allium* seed seem to point in the direction of a general physiological change which indirectly affects the chromosomes.

SUMMARY

Chromosomal aberrations appearing in the primary divisions of root tip cells in several varieties of *Allium* have been analyzed. Aging of the seed was

found to result in an increase in the number of aberrant cells. Irradiation of the dry seed also causes an increase in the number of aberrations. No correlation was found between the age of the seed and its radio-sensitivity, indicating that possibly two different mechanisms may be involved.

Delayed germination of seeds following irradiation causes a significant increase in the number of aberrations except in cases where fresh seed is used. The number of aberrations is also found to be greater

when the moisture content of the seeds is increased during the period of delayed germination.

In addition to the aberrations arising as the result of "direct hits" following irradiation, it is pointed out that physiological factors such as age of seed and moisture content are of importance in bringing about structural changes in the chromosomes.

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THE TELOME THEORY AND THE ORIGIN OF THE STAMEN¹

Carl L. Wilson

It is becoming widely recognized that the generally held concept of the essential organs of the flower as modified foliar appendages rests upon a most insecure foundation. This view of the nature of the carpel and the stamen, which may be termed the "classical" theory of the fundamental nature of the floral organs, has prevailed since the time of Goethe and A. P. De Candolle, and has been supported by many workers since that time (Arber, 1937). Certain aspects of vascularization of the carpel may be adduced in support of this point of view, but these facts may also be interpreted merely as indicating parallel development in the relationship of leaves and floral organs; we must delve deeper to understand the nature of appendages in general.

Information has become available in recent years which will permit of a more plausible hypothesis of the fundamental nature, not only of the plant body of the higher vascular plant but also of the flower

itself. Studies on fossil remains of early and simple land plants show organisms with no differentiation of leaf and axis, but rather a simple and commonly dichotomously branched body of indeterminate growth; certain of the terminal branches, the fertile telomes, bore terminal and solitary sporangia, while other branches were sterile. Information derived from such early land plants, from the ferns and fern-like plants of the Paleozoic, and from modern ferns has shown that the fern leaf, and that of the Pteropsida in general, may well have developed from a dichotomous branch system which has become webbed and flattened and from sympodial development has moved to monopodial growth with lateral branches on a main axis. No extensive account of the telome theory, which describes the plant body in terms of a branching axis, will be attempted here; the reader is referred to Bower (1935) and Eames (1936).

In attempting to account for the origin of the flower on the basis of the telome theory, we may postulate that the sporangium-bearing branches in ancient vascular plants were at first photosynthetic,

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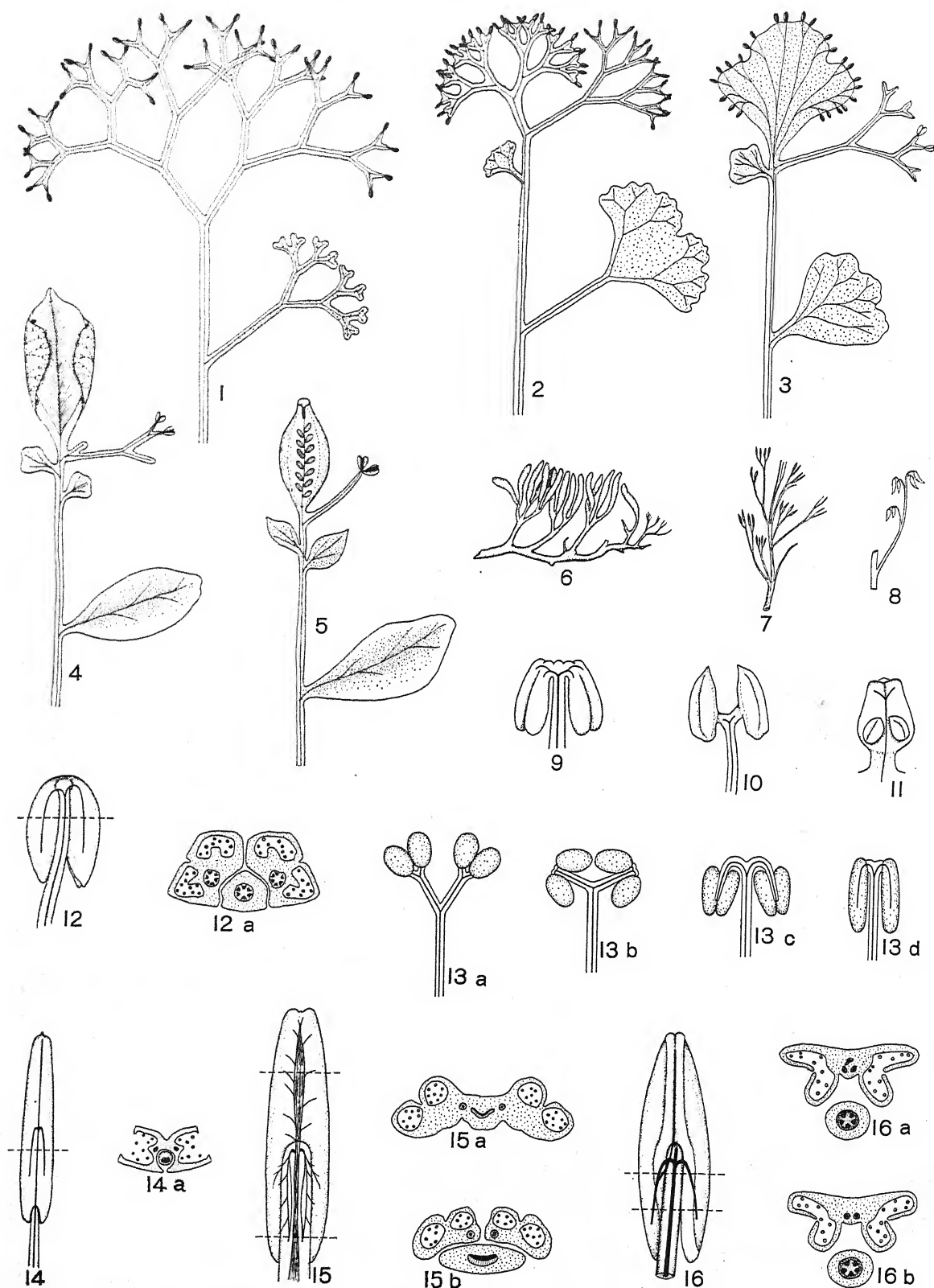


Fig. 1-16.—Fig. 1-5. Hypothetical reconstruction of the evolution of carpel, stamen, and leaf, on the basis of the telome theory.—Fig. 6. *Protopteridium hostimense*, sporangia.—Fig. 7. *Pseudosporochnus Krejci*, sporangial cluster.—

but that later this process became largely or entirely restricted to the forerunners of the modern leaf; the processes of reproduction and food manufacture in the ancestors of the modern flowering plant were early separated (fig. 1). Fertile branch systems of two kinds became distinguished, and following such processes as webbing, fusion, and reduction, one of these became the carpel, the other the stamen. Evidence bearing upon the evolution of these floral organs may be found in the papers by Hunt (1937) and Wilson (1937). The evolution of the terminal portions of the plant body is shown schematically in figures 2 to 5. The primitive foliar organ is shown to have developed through stages including flattening, webbing, and fusion of sterile telomes. Similarly, a group of fertile telomes became webbed and fused to form the carpel, which later folded along the margins, enclosing the sporangia. The stamen, likewise, is the result of reduction from a system of dichotomously branched fertile telomes, or even from a portion of such a system. From such a branch system the stamen undoubtedly evolved in different ways; in the scheme shown here, the ultimate fertile telomes are first shown to fuse in pairs (fig. 2, 3); then following reduction or fusion of the telome stalks, the paired sporangia became aggregated into groups of four, a prototype of the modern anther (fig. 4, 5). The remaining telomes of the system first became sterile, and finally disappeared.

Since our knowledge of the early forms of plant life of the Paleozoic shows that spore-producing structures antedated any differentiation of leaf and stem, the problem of the stamen then becomes not how a stamen may have become modified from a leaf but how a stamen may have arisen from a branch system possessing terminal sporangia, a system existing before leaves had evolved.

We have indicated the possible origin of the stamen, together with other organs of the plant, from a highly reduced branch system; it now remains to determine whether this structure has retained in its modern form any evidence of its ancestral history. In consideration of the undoubted great age of the race of angiosperms, of the ephemeral nature of this organ, and of the great reduction which the sporangial branches of ancient plants have undergone, it is too much to expect that unimpeachable proof will be found in the external morphology or internal structure of the stamen which will determine for all time the problem of the origin of this structure. A new method of approach to the problem has been used by Satina and Blakeslee (1941) in an investigation of the histogens which give rise to the leaf and floral organs in periclinal chimeras of *Datura Stramonium*. They found that the ontogeny of the

stamen resembles that of the shoot apex rather than that of leaf, sepal, or petal, and conclude that the stamen is a reduced axis. Aside from such studies as these, the chief lines of evidence, for the present at least, must lie in the vascularization of the stamen—of the filament and connective. A preliminary and by no means exhaustive survey of the nature and direction of the course of the vascular bundles of stamens makes possible an estimate that in probably 95 per cent of angiosperms the stamen is supplied with a single vascular bundle which traverses the filament. This bundle may end at the base of the anther or may ascend the connective for some distance, to end blindly at a greater or lesser distance from the apex. In certain other forms deviations from this general plan exist which possibly may be of significance in a consideration of stamen origin and evolution.

In any discussion of stamen phylogeny it should be emphasized that the stamen, as well as the carpel and leaf, may well have originated not only at different times but also along different lines. In the hypothetical reconstruction of the flower shown in this paper, a four-celled anther is shown to have arisen from a branch system by sterilization and reduction. The anther thus constituted may have been erect on the telome stalk, or inverted. That such branch systems existed is well known. Figures 6 and 7, from Kräusel and Weyland (1933), illustrates sporangia borne erect on dichotomous branches. This condition was common, not only in the Psilophytales, but also, in a somewhat modified form, in a great series of plants more complex than these; dichotomy, or terminal sporangia, or both, seems to have existed as a general trend throughout the middle and later Paleozoic. Figure 8 is a portion of a well-known restoration of *Psilophyton princeps* from Dawson (1888). The nature of the reproductive structures of this plant as described by Dawson (1871) is entirely confirmed by Halle (1916) who, however, refers the fructifications of the plant to the genus *Dawsonites*. It is probable that the sporangia were borne in pairs, at the apices of fertile telomes, and that the fertile branch system divided dichotomously several times. The sporangia were lax or pendulous, tending to bend back upon the fertile telome stalks which bore them. This tendency is carried further in *Dawsonites bohemicus* as illustrated by Kräusel and Weyland (1933). The sporangia of this poorly understood fossil appear to be strongly reflexed and perhaps fused with the stalks which bear them. Such pendulous sporangia, fused with their sporangial stalks, could well have given rise to those types of anthers in modern forms which appear to be borne marginally rather than terminally. A reduction of the telomes forming the

Fig. 8. *Psilophyton princeps*, portion of fruiting structure.—Fig. 9. Hypothetical primitive stamen as reduced from a double dichotomy.—Fig. 10. Stamen and vascularization, *Tilia americana*.—Fig. 11. Single stamen, *Penaea mucronata*.—Fig. 12, 12a. *Enallagma megaphylla*, entire stamen and cross section.—Fig. 13a-d. Diagrams to show origin of connective in stamen in which the anther is suspended.—Fig. 14, 14a. Stamen and cross section, *Bauhinia mollicella*.—Fig. 15, 15a, 15b. *Passiflora vitifolia*, stamen and cross sections at levels indicated.—Fig. 16, 16a, 16b. *Cobaea scandens*, stamen and cross sections.

last two dichotomies in such a form as Dawson's *Psilophyton princeps* may well have given rise to the hypothetical stamen diagrammed in figure 9, which approximates in many respects the condition found in certain modern stamens.

Since such forms existed, and may have represented strong trends in ancient plants, it should be permissible to interpret certain conditions found in living species in the light of the past. While there is considerable variation in the form of the small stamens of *Tilia americana* L., a number in each flower assume the shape shown in figure 10. The filament tends to bifurcate at the summit, each branch bearing a pair of sporangia. The single vascular bundle occasionally fades out at the point of division of the filament, but also commonly divides, each branch passing for a short distance into the filament branches. This condition may well represent a double dichotomy of a branch system, here reduced to a single dichotomy. A similar condition is also to be found in the stamen of *Penaea mucronata* L. (fig. 11), in which the paired sporangia are located toward the base of a well developed connective. The single bundle enters the filament and passes to the top of the connective, dichotomizing at the apex. The sporangia in this species may have migrated to the base of the connective, or the connective may have become greatly enlarged.

The vascular supply of the stamen of *Enallagma megaphylla* (D.Sm.) Standl. is clearly dichotomous. The anther is essentially of the suspended type (fig. 12), attached to the filament but a short distance below the apex. At the point of attachment the filament bundle dichotomizes, sending two bundles downward in the connective almost to the apices of the anther halves. This condition is clearly revealed by a cross section of the entire anther (fig. 12a) taken from the region indicated by the dotted lines. It should be noted that here the paired sporangia on either side of the filament remain discrete; the walls between the pairs do not break down, and, as in many other species, the anther contains four pollen sacs (sporangia) at maturity. The diagrams in figure 13, a-d, show how this condition could have arisen. The stalks of two pairs of sporangia first became reduced, so that the sporangia were almost sessile and were contiguous. Each pair of sporangia then became folded back upon a penultimate unit of the branch system and became fused with this unit. If these latter units then became pendulous, the condition exhibited by *Enallagma* would have arisen.

Extensive modifications of this dichotomous system may possibly be illustrated by the course of the staminal vascular tissues in *Bauhinia mollicella* Blake (fig. 14),² *Passiflora vitifolia* HBK. (fig. 15) and *Cobaea scandens* Cav. (fig. 16). In all of these forms the filament is attached to the anther midway between the apex and base of the anther; in *Bauhinia* and *Passiflora* the filament is dorsi-fixed, in

Cobaea the filament is attached on the ventral or adaxial side. The general trend of vascularization is the same in all; a single vascular bundle passes up the filament and divides into three parts in the connective in the region of attachment of the filament. Two of these branches proceed downward in the connective on either side, the third passes upward, apparently as a continuation of the main bundle of the filament, except in *Cobaea*, in which it appears as a branch of one of the downwardly directed bundles. Cross sections of the anthers are shown in figures 14a, 15a, 15b, 16a, 16b, taken from the regions indicated by the dotted lines. Several interpretations of this condition as a modification of the dichotomously branching pattern may be advanced, and one of these is diagrammed in figure 17, a-e. In figure 17a is shown a terminal branch system consisting of four fertile telomes, in which the two penultimate units of the system have become reduced to the extent that the telomes arise from the same point. In 17b the telomes have become reflexed and the sporangial stalks partly fused. In 17c and 17d fusion has proceeded farther; the paired telomes on either side of the main stalk of the system have fused together, and these in turn have become partly fused with the basal stalk. If, now, the sporangial pairs should become compressed and elongate acropetally, they would become adherent to the stalks on which they are borne, and the vascular bundles would come to lie in the position shown in figure 17e.

✓The problem of the stamen with three traces has always attracted considerable interest, and Eames (1931) cites certain families in which this condition occurs. This situation may well be primitive, as Eames states, and the three-trace system may have arisen through a series of modifications of a dichotomous branch system as shown in figure 18, a-d. In figure 18a is shown the system previous to reduction. In 18b the basal stalk of the system has dropped out, and six central units of the system are about to fuse. In 18c complete fusion has taken place, accompanied by reduction and sterilization which has led to the disappearance of the central sporangia of the branch system. Further sterilization and reduction would result in the three-trace system shown in figure 18d.

The staminal vascularization of *Fremontodendron mexicanum* Dav. (*Fremontia californica* Torr. var. *mexicana* Jepson)³ and *Cassia undulata* Benth. may well represent reductions from this hypothetical scheme. *Fremontodendron* (fig. 19) possesses a true three-trace system, each bundle arising independently from the stele. The central trace traverses the entire length of the connective, which extends into a point beyond the paired sporangia. Here the central bundle dichotomizes. The lateral traces each give off a bundle at the base of the anther, and these bundles, branching somewhat, pass downward into the connectives of the paired thecae (fig. 19b). As the lateral traces move upward in the connective

² Ex. Herb. New York Bot. Gard., Killip and Smith 16525. Acknowledgment is due to the authorities of the Garden for herbarium material of this and other species examined in the course of this study.

³ For preserved material of this species the writer is indebted to Professor I. L. Wiggans, of Leland Stanford Junior University.

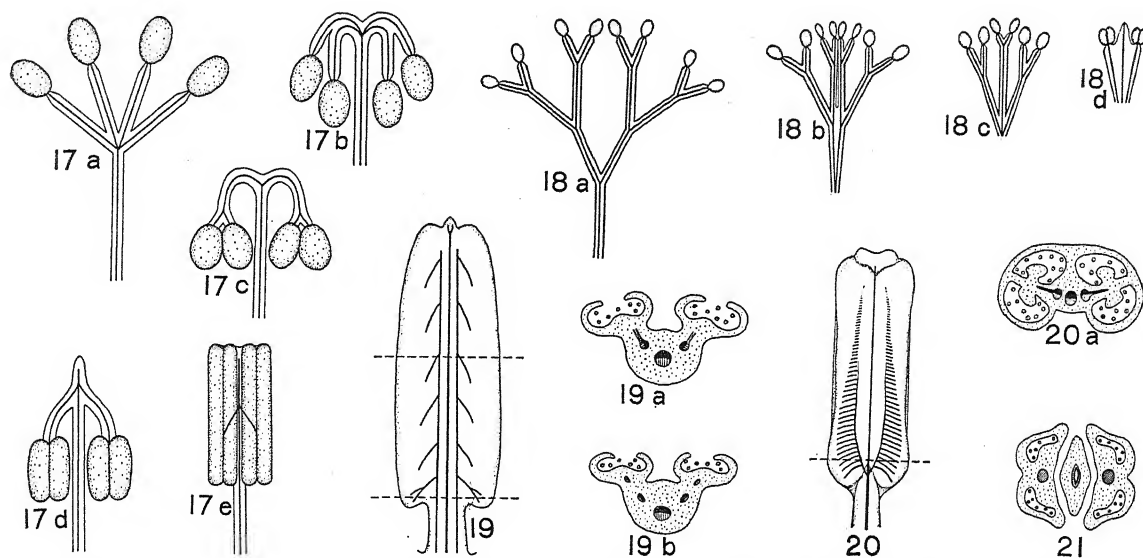


Fig. 17-21.—Fig. 17a-e. Hypothetical course of evolution of a stamen with marginal sporangia.—Fig. 18a-d. Hypothetical origin of a three-trace stamen from a dichotomous branch system.—Fig. 19, 19a, 19b. *Fremontodendron mexicanum*, stamen and cross sections.—Fig. 20, 20a. *Cassia undulata*, stamen and cross section.—Fig. 21. Cross section of stamen of *Cassia marginata* close to apex of anther.

they give off a series of branches, like the veins of a feather, which pass into the tissue of the connectives on either side (fig. 19a). While many aspects of vascularization in this stamen remain to be explained, the nature and course of the main bundles may be found to be most significant in connection with the problem of stamen phylogeny after the stamens of a great series of forms have been studied from a comparative standpoint. The three main bundles traversing the connective may be held to represent the three bundles shown in figure 18d, and the bifurcation at the apex of the central trace a remnant of the condition shown at the apex of the central bundle of figure 18c, after the sporangia have disappeared from that portion of the branch system.

In the larger of the two sets of stamens of *Cassia undulata* (fig. 20) the course of the vascular bundles in the connective is similar to that of *Fremontodendron*. The filament contains but one large horseshoe-shaped bundle, possibly the result of the fusion of three. This bundle divides into three at about the point of insertion of the filament on the anther. The central trace does not dichotomize but passes upward in the center of the connective, to fade out at the very apex of the anther. The lateral bundles are at first quite strong (fig. 20a), giving off connections into the tissue of the connective on either side; later, they become weaker and disappear at a level below that of the disappearance of the central bundle. Certain other species of *Cassia* show this same vascular plan with some modifications, a plan which may be looked upon as the result of the partial fusion of the bundles of an originally three-trace system. Among the more interesting deviations from this plan is that found in *Cassia marginata* Willd. (fig. 21). The three main bundles closely approach

each other near the top of the anther. At this point the anther divides into three parts, a bilocular region on either side with an apical projection between. The central bundle remains in this projection, while the lateral ones pass into the connectives of the paired sporangia on either side. This condition is essentially that found in the hypothetical reconstruction shown in figure 18d.

The conditions just described by no means exhaust the deviations from the common condition of a single unbranched bundle supply in the filament and connective. In many species these take the form of one or several branches arising irregularly from this central bundle at various points in the filament and connective; while these conditions may be related to reduction from the three-trace system, either reduction has proceeded too far, or intermediate stages are lacking, so that a definite interpretation is lacking in these cases.

Discussion.—The present paper is by no means intended as a definitive and final pronouncement upon the origin and evolution of the stamen. It is, rather, in the nature of a progress report, calling attention to certain aspects of stamen vascularization which may be of significance in a renewed attempt to understand the nature of the stamen in the light of fresh viewpoints arising from an increased knowledge of ancient plants. The evidence thus far available for the origin of the stamen in the light of the telome theory is by no means conclusive. On the other hand it seems desirable to emphasize the fact that the classical theory of the origin of the floral parts has come to us largely as the result of certain metaphysical concepts of Goethe (Arber, 1937), and is supported chiefly by evidence derived from teratological phenomena, a type of evidence which has long fallen into disrepute.

Here, then, is a concept of the stamen different from that of a modified foliar appendage; moreover, acceptance of the telome theory with respect to the stamen as a working hypothesis does not invalidate our generally held view of the flower as an axis with highly modified and specialized leaf-like appendages; we may now regard the stamen, and the carpel as well, not as modified leaves but as reduced branch systems, homologous with leaves of a similar origin. Floral organs resemble leaves, and their vascularization is similar because they have evolved in a similar way.

It is possible that the nature of the primitive stamen is still unknown, although it is probable that a synangium of four sporangia is a very ancient structure and was derived from the reproductive structures of ancient lines which gave rise to such groups as the flowering plants, the ferns, seed ferns, and the Cordaitales. Since the nature of the primitive stamen is not positively known, it is not possible to interpret finally all of the various aspects of form in the modern stamen. The nature of the connective, for example, is not clear; a working explanation may be reached, however, after consideration of the lax or pendulous nature of the sporangia in certain ancient forms. If these became reflexed and fused with their telome stalks, the marginal position of the pollen sacs with respect to the connective in great numbers of species may be explained. Undoubtedly, also, secondary vascular bundles, whose positions are not significant from a phylogenetic viewpoint, have arisen in connection with nutrition and translocation. It is possible that the vascular anatomy of the stamen may never be interpreted to the degree, for instance, that we now understand the relation between carpel and leaf. In the flower as a whole, such problems as the cauline ovule, free central placentation, epigyny, the reduction and disappearance of floral parts, the interpretation of vestigial structures, the determination of phylogenetic relationships—these and others have been solved or partly solved by the anatomical method.

This, however, is essentially comparative in its approach; structures in a large range of forms have been studied before interpretations could be made and conclusions drawn. It must be likewise with the stamen, with the added great difficulty that this organ is much more reduced, both externally and with respect to its vascular tissues, than any other organ of the flower.

SUMMARY

The "classical" theory of the nature of the floral organs as modified foliar appendages is attacked, especially from the standpoint of its inadequacy in explaining the origin of the stamen. A concept of the evolution of the stamen based upon the telome theory is presented. This viewpoint considers that the anther is a synangium of four sporangia, and that this synangium, together with the filament and connective, has arisen from a dichotomous branch system with terminal sporangia, following such processes as fusion and reduction of the fertile telomes (sporangia and their stalks) and other parts of such a system. The theory receives a measure of support from paleobotanical studies upon the reproductive structures of certain ancient and primitive plants of the Paleozoic. It is further supported by the staminal vascularization of certain selected living angiosperms.

It is suggested that the vascular bundles and their connections in these forms may represent vestiges of the vascularization of such ancient branch systems. An understanding of stamen morphology may be attained by considering the filament as the basal part of such a system, the connective consisting of modified subsidiary branches to which the reflexed sporangia have become fused. The vascularization of the three-trace stamen may similarly be derived from that of a dichotomous branch system following extensive reductions and fusions.

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AUXIN CONTENT OF MAIZE KERNELS DURING ONTOGENY, FROM PLANTS OF VARYING HETEROTIC VIGOR ¹

G. S. Avery, Jr., J. Berger, and B. Shalucha

A METHOD involving alkaline hydrolysis has been developed for the rapid and total extraction of auxin from dormant endosperms of maize and other cereal grasses (Avery, Berger and Shalucha, 1941). Upon preliminary tests this method was found to give extremely high yields of auxin from freshly harvested immature corn kernels. Other tests showed that these yields were much higher than those obtainable with repeated alcohol or ether extractions, a fact previously established for dormant corn. The method was, consequently, regarded suitable for studies on the rate of auxin accumulation in corn kernels during their ontogeny. The procedure arbitrarily differentiates between free auxin and precursors of auxin, the former being that detectable before alkaline hydrolysis, the latter the increase obtainable upon alkaline hydrolysis. It is noteworthy that while alkaline hydrolysis has not proved satisfactory for a number of green tissues on which it has been tested, it is highly effective as a method of extracting auxin from growing kernels of corn.

The objects of this study were as follows: (1) to determine the extent of auxin storage at intervals in the ontogeny of the kernel; (2) to determine whether marked differences in vegetative vigor of plants are reflected in equally marked differences in the auxin content of the kernels they bear—thus the study concerned the possible effect of growth vigor on auxin accumulation in storage tissue, not the effect of auxin on growth. In the course of these experiments kernels from vigorous hybrids and polyploids of varied genetic origin and makeup were investigated, and compared with kernels from less vigorous inbreds. Although some of the evidence presented might indicate a relationship between heredity and auxin storage, the experiments were not designed with this in view, and evidence along such lines is considered inadequate for any generalizations.

Previous work on the ontogeny of maize (Laibach and Meyer, 1935) and on the growth of *Epilobium* hybrids (Graze and Schlenker, 1936) in relation to auxin content was done before quantitative extraction procedures and refined assay methods were available. Goodwin (1937) has reported that by the diffusion technique the relative concentration of auxin in leaves of *Solidago sempervirens* is consistently higher than in *S. rugosa* or the hybrid between them.

Hatcher and Gregory (1941), employing water as a solvent for extraction of auxin from kernels of winter rye during their ontogeny, report auxin content to increase rapidly from a very low value at time of pollination to a maximum five to six weeks later.

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We are grateful to Miss Mary Hickox for carrying out a considerable number of assays, and for her general interest in the problem.

At maturity the content had decreased to ca. 12 per cent of the maximum.

MATERIALS AND METHODS.—The stages of development of the corn kernels which were used and the ears from which they were taken, are shown in figure 1. Table 1 gives the varieties etc. used, dry weights, per cent dry matter and source. It is a pleasure to acknowledge the cooperation of E. B. Clark, D. F. Jones, John Shafer and L. F. Randolph in supplying at intervals the freshly harvested ears of corn in various stages of development. All plants were field grown.

Whole ears of corn were harvested in every instance and transported by motor car to the laboratory. The only exception was the Cornell material

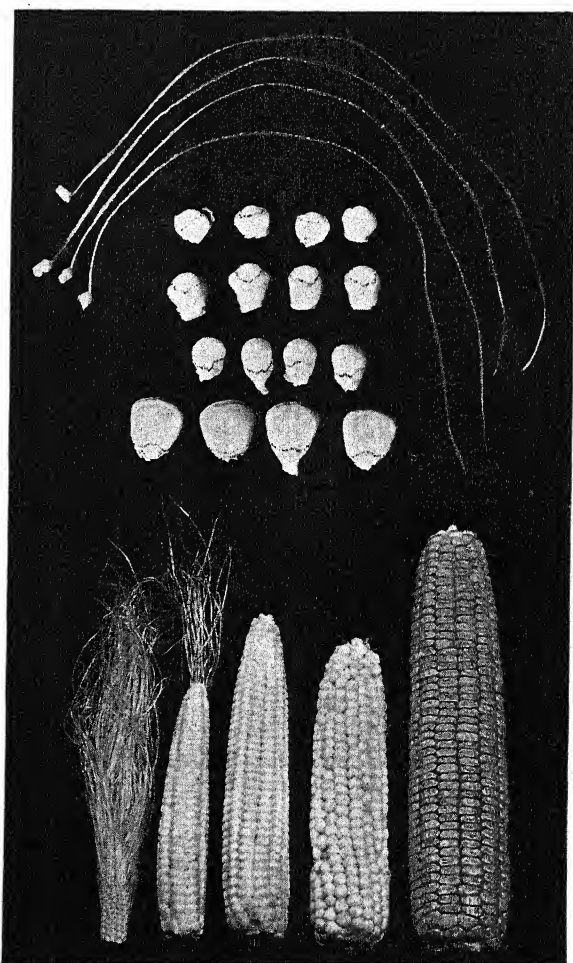


Fig. 1. Above, corn kernels at the stages assayed for auxin precursor and free auxin. Below, ears from which the above kernels were taken; stages left to right, silk, blister, milk, dough, dormant.

TABLE 1. Per cent dry matter and dry weights of corn kernels at the different stages of development studied. The first eight samples were obtained from E. B. Clark, Associated Seed Growers, Milford, Conn., and all possess sugary endosperms. The last three samples were obtained from D. F. Jones, Conn. Agric. Exper. Sta., New Haven, Conn., and possess a soft dent endosperm, characterized by a small amount of corneous endosperm and a larger proportion of soft starch.

Variety or pedigree	Dry weight of kernel in milligrams and per cent dry weight at the stages of development indicated						
	Silk	Blister	Early milk	Late milk	Early dough	Late dough	Dormant
Golden Cross Bantam	120	180	185	190	220
	31%	38%	46%	46%	89%
Country Gentleman	4	34	..	75	150
	12%	35%	..	40%	82%
Inbred 75	42	..	110	130	130	210
	..	26%	..	36%	40%	42%	86%
Hybrid 75 × 77	35	..	170	160	200	280
	..	18%	..	37%	36%	49%	89%
Inbred 77	25	33	110	120	..	190
	..	15%	19%	28%	27%	..	74%
Inbred 14	14	70	140	..	170	190
	..	11%	31%	37%	..	39%	83%
Hybrid 14 × 5	40	..	110	110	..	180
	..	21%	..	31%	36%	..	84%
Inbred 5	30	80	80	110	..	200
	..	18%	26%	30%	33%	..	65%
Inbred C-14	3	18	19	120	..	150	270
	13%	15%	13%	38%	..	44%	82%
Hybrid 677 × C-14	6	50	..	120	220	370
	..	8%	18%	..	30%	48%	88%
Inbred 677	4	7	22	150	400
	16%	9%	12%	39%	81%

which was brought directly by train from Ithaca. The latter was approximately eighteen hours from field to laboratory, the former only two hours. Kernels from each ear of corn were assayed immediately, or whole ears were kept for not more than three to four days at 2°C. until extractions and assays could be made; proper precautions were taken to prevent desiccation. It was of course physically impossible to make all auxin assays on the day of harvest, since it would have involved 100 to 150 dozen test plants.

Crushed tissues were never stored, i.e., only the whole ears were stored, and such storage might be suspected of resulting in variable alterations in

yield, but this did not prove to be the case. Fresh corn, stored as above for as long as one week, did not show any appreciable increase or decrease in total yield, or in proportion of precursor to free auxin.

The methods of auxin extraction and assay used in this study have been reported in full (Avery, Berger, and Shalucha, 1941). Because of the exploratory nature of the study and the large number of assays involved, only six *Avena* test plants were used per dilution (instead of the usual dozen). However, this made possible the testing of a number of dilutions at one time, and thus expedited the finding

TABLE 2. Sample calculation of auxin yields for kernels of the variety Country Gentleman in the late milk stage.

Treatment	Total volume in cc. of agar- extract mixture from 1 gm. fresh tissue	<i>Avena</i> curvature in degrees (deseeded method)	Millions of TDC or tens of micrograms of indoleacetic acid		
			Per gram fresh wt.	Per gram dry wt.	Per kernel
Water extraction (free auxin)	200	13.8
	400	7.3	0.29	0.7	0.05
Alkali hydrolyzed (total auxin)	4000	29.7
	8000	19.0 ^a	15.0	38.0	2.9

^a Degrees curvature × agar volume in cc. × 100

Using the formula $\text{TDC/gram} = \frac{\text{Degrees curvature} \times \text{agar volume in cc.} \times 100}{\text{Weight of tissue in grams}}$, we have in this case $19 \times 8000 \times$

$100 = 15$ million TDC/gram fresh weight. The values 38 and 2.9 are calculated from the above and the data in table 1: per cent dry weight of kernel = 40, and dry weight of kernel = 75 mg.

of dilutions which gave curvatures in the proportionality range. Duplicate assays on representative

samples showed experimental variation of approximately 20 per cent, which is the normal variation in the *Avena* technique.

Values for "free" auxin content were obtained as follows: kernels were removed from the central portion of the ear and ground with a pestle; 0.5 gm. of this tissue was extracted with 10 cc. of water at approximately 25°C. for fifteen minutes, and the clear centrifugate of this suspension was assayed by the deseeded *Avena* method.

Values for "total" auxin content (auxin plus hydrolyzed auxin precursor) were obtained as follows: 0.25 gm. of ground tissue obtained as above was heated in 25 cc. of M/20 borate buffer, pH 9.6, at 120°C. for fifteen minutes. This was then neutralized to pH 6, and the clear centrifugate assayed.

The auxin precursor present in a given sample of tissue is determined by subtracting the amount of free auxin from the total auxin content after hydrolysis. The so-called precursor is an as yet unidentified compound (or compounds) which upon alkaline hydrolysis yields auxin. There is no appreciable conversion of precursor (less than 2 per cent) to free auxin during the five-hour deseeded *Avena* assay. Two per cent of the precursor yield is rarely of sufficient magnitude to affect the significance of the free auxin values. A simple calculation by the reader will make it clear wherever this has occurred.

TOTAL AUXIN IN WELL-KNOWN VARIETIES OF CORN DURING ONTOGENY.—Figure 2 shows the quantitative occurrence of auxin and auxin precursor in kernels of the varieties Country Gentleman and Golden Cross Bantam at different stages of development. The most striking fact is the overwhelming predominance of auxin precursor over auxin. The free auxin is never greater than 12 per cent of the total, and at the peak stages it constitutes not more than 3 to 6 per cent of the total.

On a dry weight basis it may be noted that the peak of total auxin content occurs at the blister stage of Country Gentleman, and not until the late milk stage for Golden Cross Bantam; whereas on a per kernel basis both varieties reach their peak at the late milk stage.

In the case of Country Gentleman both total auxin content and dry matter increase approximately ten-fold from silk to blister stage. From this peak to dormancy, dry weight increases another four-fold and total auxin concentration decreases to one-fourth its peak value. It appears, therefore, that in Country Gentleman auxin and dry matter increase together up to the blister stage, and, from this stage to dormancy, dry matter continues to accumulate, but total auxin apparently does not.

TOTAL AUXIN IN CORN INBREDS AND HYBRIDS DURING ONTOGENY.—The objects of the experiments reported in this section were: (a) to study the rate of auxin accumulation in kernels of inbreds and hybrids during their ontogeny, and (b) to determine whether marked differences in vigor of plants are reflected in equally marked differences in the auxin content of the kernels they bear. It should be pointed

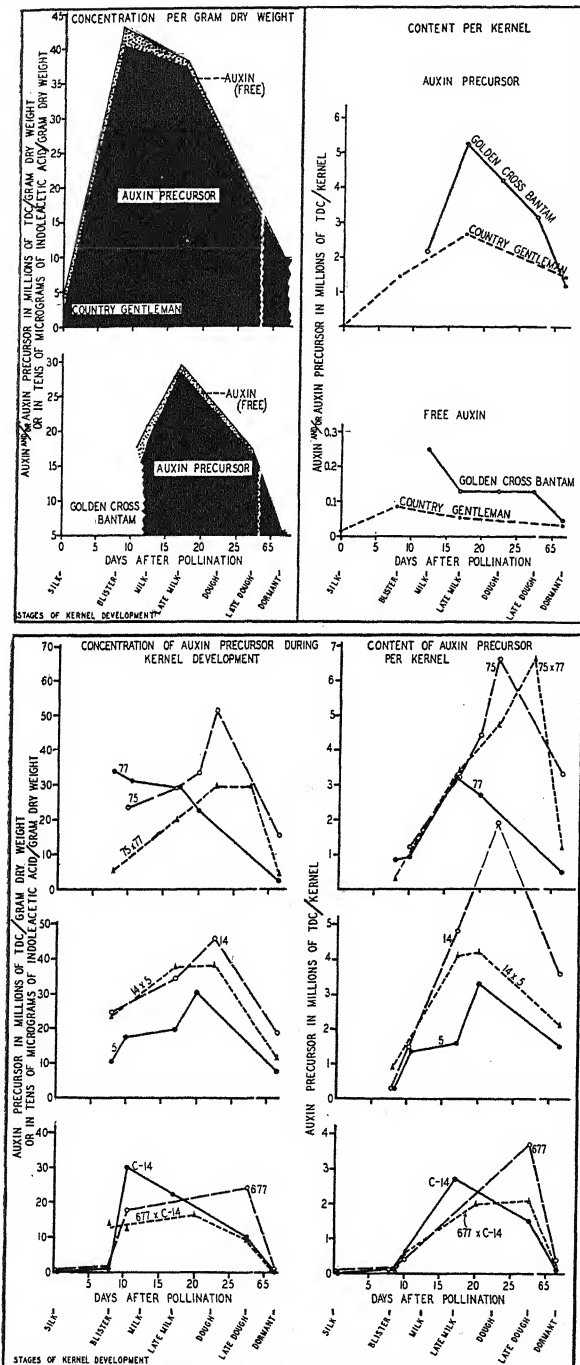


Fig. 2-3.—Fig. 2 (above). Occurrence of auxin precursor and free auxin in kernels of "Country Gentleman" corn from time of pollination (silk stage) to maturity; "Golden Cross Bantam" from the milk stage to maturity. Both of these varieties possess a sugary endosperm.—Fig. 3 (below). Occurrence of auxin precursor in kernels of hybrid and inbred corn at different stages in development. Pedigrees 77, 75, 14 and 5 possess sugary endosperm; C-14 and 677 have starchy endosperm.

out that all the kernels from hybrid plants, reported in this part of the study, were themselves F_2 rather than F_1 kernels. This should be noted in contrast to the data in table 4.

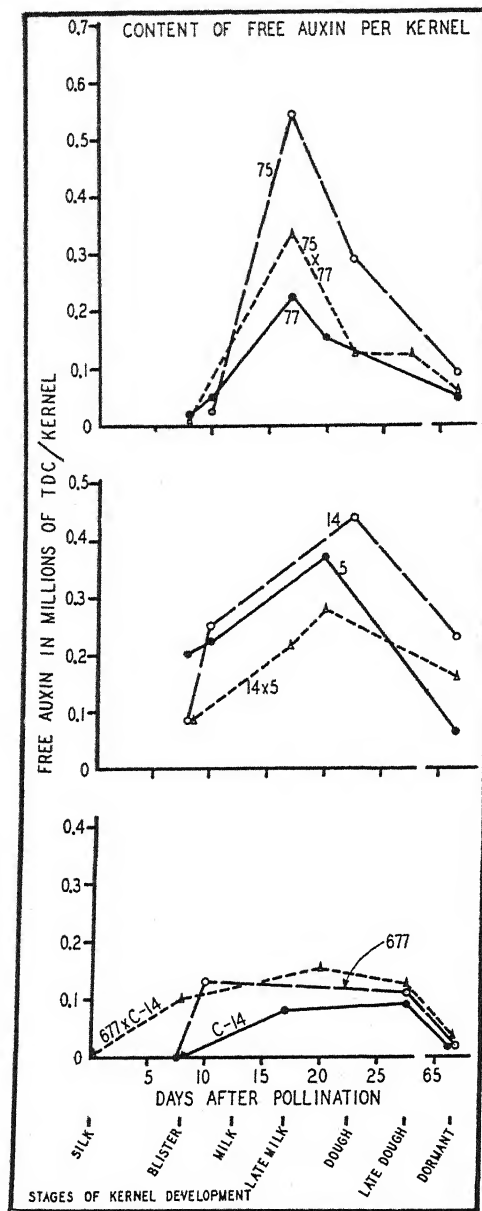


Fig. 4. Occurrence of free auxin in kernels of hybrid and inbred corn at different stages in development. See legend of figure 3 for endosperm types.

In the graphs of figure 3, auxin precursor of three sets of inbreds and hybrids is plotted on a concentration and content basis. The former represents concentration per unit weight of tissue and the latter the absolute amount per kernel. In figure 4, values for free auxin are plotted on a content basis. The following points may be noted from these graphs:

(1) During development of the kernels, the peak auxin precursor and free auxin content is generally reached in the dough stage. At this point, all the corn samples with sugary endosperm contained from thirty to fifty million TDC per gram dry weight of total auxin (equivalent to 0.3 to 0.5 mg. of indoleacetic acid per gram of tissue), while samples with starchy endosperm contained fifteen to thirty million. In two instances (inbred WF, not reported here, and hybrid 487×479 , table 4), a total auxin yield of over eighty-four million TDC per gram dry weight was obtained. These values represent, with one possible exception (Linsner, 1940), the highest concentrations of total auxin and auxin precursor known to occur in plant tissues.

(2) There appears to be no fixed relationship between the absolute amounts of auxin precursor and free auxin accumulated in the kernels, except that the precursor always constitutes the major portion of the total obtainable at any time. In general, the proportion of free auxin is greater in the early than in the late stages of development. The curves for both the precursor and free auxin yields are of approximately the same shape throughout ontogeny, and the respective yields are of very nearly the same magnitude for a given endosperm type.

(3) It was pointed out that in the variety Country Gentleman, changes in total auxin concentration and dry matter content were closely associated with one another. In the inbreds and hybrids, both dry matter and total auxin concentration increase up to peak yields of the latter, but not always in constant proportion; nor are increases in free auxin content always proportionately related to changes in dry weight. From the auxin peak to maturity, the actual content of auxin per kernel decreases while dry matter continues to be accumulated.

To determine whether drying in itself was causal in decreasing the yield obtainable at dormancy, kernels of three different lines were air dried at their peak of total auxin concentration; re-assays were made after three months. In no instance was the loss of auxin precursor greater than 25 per cent, and the free auxin values were slightly higher than the original assays. Inasmuch as kernels allowed to mature normally lose about 80 per cent of their auxin, on the average, it appears that in addition to the drying of the kernels, other factors must be responsible for the markedly decreased auxin yields obtainable at maturity.

(4) In no instance do the auxin yields of the hybrids significantly exceed those of the inbreds, even though the hybrids show heterosis (table 3). This may be due to the material studied: the hybrid kernels tested were those growing on F_1 plants, and they, therefore, represented the F_2 generation. However, the F_2 kernels were produced on vigorous F_1 plants, and, if the vigor of a parent were accompanied by increased auxin production, one would think that the hybrid kernels might reflect this vigor in accumulating a greater auxin content. Preliminary results obtained with kernels of the F_1 generation

TABLE 3. *Data on vegetative and yield vigor of inbred and hybrid corn.*

Pedigree	Vegetative growth	Yield	
		Ave. ears per stalk	Ave. yield of dry shelled grain in bushels per acre
677	65% of hybrid	1.0	52
C-14	65% of hybrid	1.0	29
677 × C-14 ^a	1.0	105
Ave. number of kernels per ear			
14	50% of hybrid	1.5	284
5	90% of hybrid	1.5	358
14 × 5	1.0	700
75	50% of hybrid	2.0	205
77	75% of hybrid	1.5	256
75 × 77 ^b	2.5	600

^a Dr. D. F. Jones, of the Connecticut Agricultural Experiment Station, writes regarding 677 × C-14: "We think this is one of our most vigorous hybrids, when considered for its early production of grain."

^b Mr. E. B. Clark of the Associated Seed Growers, Milford, Conn., writes: "In the 75 × 77 hybrid we probably get a higher number of ears per plant, on the average, than with any other hybrid now in commercial production."

(presented in table 4), also failed to show any regular increases in auxin yields over the better of the parents.

If the auxin which accumulates in the kernels is calculated on a *per plant* basis, the hybrid considerably excels either parent in the case of 75 and 77 (fig. 5). This is attributable entirely to the greater number of kernels per ear and more ears per plant in the hybrid 75 × 77. The hybrids 677 × C-14 and 14 × 5 (fig. 5) do not give a higher auxin yield per plant than the better of the parents, even though

they exhibit greater vegetative vigor (table 3). Thus the evidence indicates that the vegetative vigor of the plant is not reflected in the amount of auxin accumulated in the kernels it bears. Where yield vigor is reflected in greater auxin content per plant, the auxin concentration per unit weight of kernels is no greater than that in kernels produced by less vigorous plants.

AUXIN CONTENT OF KERNELS FROM RECIPROCAL CROSSES.—Since vegetative vigor was found not to be reflected in higher auxin content of F₂ kernels

TABLE 4. *Auxin concentration in corn inbred and hybrid kernels at the late milk stage and at dormancy. All lines were pollinated August 3, 1941, and the late milk stage auxin assays were made on samples picked seventeen days after pollination. The dormant samples were harvested on October 7, except for (468) and (468 × 479), which were harvested on September 24. The reciprocal crosses included in this table are reported to have the same hybrid vigor.*

Pedigree	Kernel characteristics	Late milk stage				Dormant stage
		Yield in millions of TDC or tens of micrograms of indole-acetic acid per gm. dry weight		Dry wt. per kernel in mgs.	Per cent dry matter	Yield of total auxin in millions of TDC/gm. dry wt.
		Auxin precursor	Free auxin			
468	Yellow dent, medium sized kernels	44	4.1	18	17	0
468 × 479	48	6.7	20	18	2.8
479 × 468	17	1.3	49	19	0.8
479	White semi-dent, large kernels	33	3.0	42	19	3.8
479 × 487	19	1.6	50	21	1.2
487 × 479	80	4.2	22	23	2.3
487	White semi-dent, small kernels	48	2.8	20	20	1.1
487 × 485	37	2.7	15	17	0.3
485 × 487	7.0	1.5	35	22	0.5
485	Yellow dent, medium sized kernels	14	1.0	28	21	0.3

(growing on F_1 plants), it was thought worthwhile to explore the situation in F_1 kernels (growing on p_1 plants). For this purpose immature and mature kernels from reciprocal crosses and parental lines were supplied by John Shafer, Jr., of Cornell Uni-

versity. The data are presented in table 4, and the following points may be noted:

(1) As regards total auxin concentration, the high values obtained at the milk stage are comparable with the peak yields of other inbreds and hybrids (fig. 2 and 3). However, the yields at dormancy are in general lower than most of those encountered in our assays of dormant maize.

(2) Free auxin and auxin precursor concentrations in hybrid kernels are not regularly greater than those of the inbreds, even though the hybrid plants produced from these kernels all exceeded the inbreds in vegetative vigor. In one instance, however, the free auxin concentration of a hybrid (468×479) clearly exceeds both inbred parents, and in another instance (487×479) the auxin precursor concentration is about double that of the high parent. Thus these results are in accord with those shown in figures 2 and 3, and indicate again that there is no clear-cut relationship between vegetative vigor of hybrids and the amount of auxin which the kernels store.

(3) In every instance in table 4, the hybrids follow the maternal parent in auxin precursor concentration at the late milk stage, but at the dormant stage they follow the paternal parent (with one exception). However, from the dry weights it may be seen that the kernels of some lines had developed more rapidly than others by the milk stage, and in all cases the heavier kernels were lower in total auxin concentration. Therefore, the mere accumulation of dry matter may be of as much as, or more significance than, inheritance in determining the concentration of auxin in the kernel. Furthermore, the auxin concentrations vary greatly in hybrid kernels from reciprocal crosses, even though the F_1 plants which later came from such kernels are reported to have the same degree of vegetative vigor. The evidence for genetic control of auxin content, although suggestive, is entirely inadequate to establish any general trends. It should be emphasized again that these experiments were designed to discover any relationship between vegetative vigor and auxin content of kernels, rather than between heredity and auxin.

AUXIN CONTENT AND POLYPLOIDY IN CORN.—Polyploidy is frequently associated with increased size of plants or certain plant organs, and it is, therefore, of interest to know whether polyploidy is in any way reflected in the amount of auxin accumulated in kernels. A preliminary investigation of this problem was made possible through the courtesy of L. F. Randolph of Cornell University, who furnished immature kernels from two series of diploid and tetraploid corn at three stages of development. The results of this study are given in table 5. It may be noted that in both diploid series, auxin precursor concentration decreases with increase in weight of kernels; this observation does not hold for the tetraploids. On a per kernel basis, precursor content remains approximately constant in both diploid series, but increases with increasing kernel weight in the tetraploids. It might appear that the diploid kernels have higher total auxin concentration than the tetraploids, but here again, as in the case of the Cornell hybrids and inbreds, high total auxin concentration

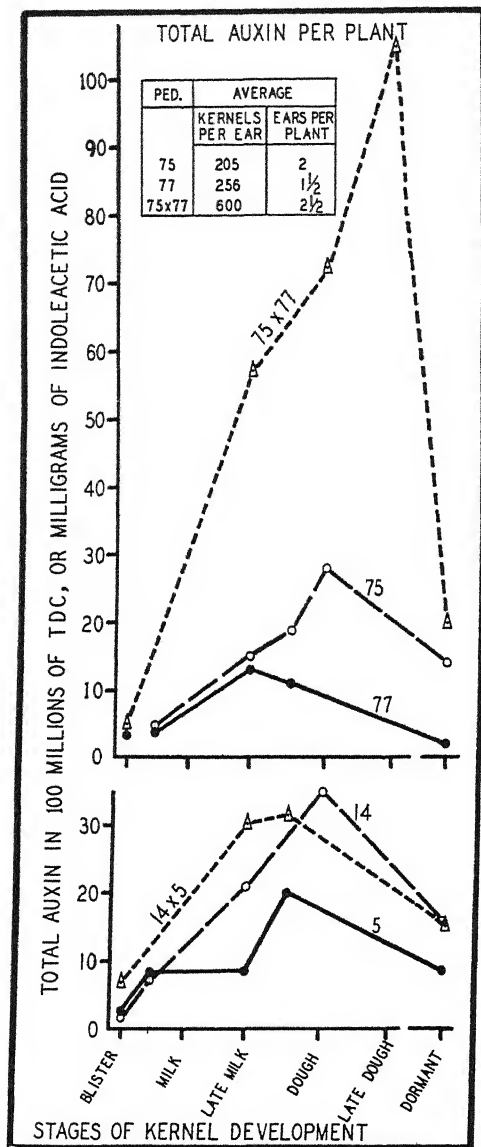


Fig. 5. Total auxin (auxin precursor plus free auxin) per plant, based on average kernel number per ear, and average number of ears per plant. Above, data on kernel and ear number are given in graph. Below, (14) averages one and one-half ears per plant and 284 kernels per ear; (5) averages one and one-half ears per plant and 358 kernels per ear; (14 \times 5) averages one ear per plant and 700 kernels per ear.

versity. The data are presented in table 4, and the following points may be noted:

(1) As regards total auxin concentration, the high values obtained at the milk stage are comparable

TABLE 5. *Auxin yields in immature kernels from polyploid corn plants. All kernels possess a flinty endosperm.*

Pedigree	Stage of development ^a	Dry weight per kernel in mgs.	Per cent dry weight	Yield in millions of T D C or tens of micrograms of indoleacetic acid			
				Per gm. dry wt.		Per kernel	
				Auxin precursor	Free auxin	Auxin precursor	Free auxin
65-2N	a	9	16	50	3.2	0.45	0.03
	b	13	23	36	4.7	0.48	0.04
	c	37	26	14	3.1	0.52	0.12
66-4N	a	25	19	15	5.0	0.37	0.12
	b	35	16	26	0.4	0.88	0.01
	c	36	19	22	1.9	0.79	0.07
J-2N	a	24	26	42	4.5	1.0	0.11
	b	43	33	29	1.2	1.2	0.05
	c	60	48	21	1.3	1.2	0.08
J-4N	a	27	19	27	2.9	0.72	0.08
	b	36	21	24	2.8	0.86	0.10
	c	76	30	24	1.7	1.9	0.13

^a a, b, c represent successively older stages in development of the corn. An approximate idea of the ages of these kernels may be had by comparing the per cent dry weight values with similar figures given in table 1; in general, the stages ranged from blister to dough. It should be noted that no dormant (mature) kernels were assayed.

is associated with low kernel weight. The above exploratory experiments show no sufficiently definite relationship between polyploidy and auxin accumulation in immature kernels to justify any general conclusions. Further work must be done.

DISCUSSION.—The primary object of the investigation was to determine the changes in the amount of auxin stored in corn kernels as they developed from the time of pollination to maturity. All the varieties studied were characterized by a rapid rise in auxin content for about three weeks (milk-dough stage), followed by a marked decrease observed at the mature (dormant) stage. This sequence of rise and fall of auxin extractable from a cereal grain during ontogeny is very similar to the one reported by Hatcher and Gregory (1941) for winter rye. These authors pointed out that the rapid decrease in auxin after the peak is apparently closely related to desiccation, whereas in the present study, at least 50 to 75 per cent of the decrease was found not to be due to mere drying of kernels.

It is interesting to note that Laibach and Meyer (1935), using alcohol extraction and lanolin paste assays, also reported higher auxin yields from corn kernels in the milk stage than from either very young or mature kernels.

It has been pointed out in this study that kernels with sugary endosperm possess more auxin than those with starchy endosperm. This agrees with results of a previous study on a number of different endosperm types assayed in the dormant stage (Avery, Berger and Shalucha, 1942).

The second object of the investigation was to determine whether marked differences in vegetative vigor of plants are reflected in equally marked differences in the auxin content of the kernels they bear. The problem was, therefore, to determine whether or not the amount of auxin stored in the

kernels is related to the vigor of the parent plant. The data obtained indicate that it is not. It is realized, however, that to establish such a negative relationship beyond question requires further experiments. These are under way.

While the vegetative vigor of corn plants does not appear to be reflected in the auxin content of the kernels, it might of course be reflected in the auxin content of the vegetative tissue. This problem cannot yet be investigated because of the lack of adequate methods for extraction of auxin from green tissues.

SUMMARY

Maize kernels were harvested at intervals during their ontogeny from the varieties Country Gentlemen, Golden Cross Bantam, and a number of pedigree inbreds and hybrids; also from polyploid plants. Free and precursor auxin were extracted by a method involving alkaline hydrolysis, and the extracts were assayed by the *Avena* technique.

At the time of pollination the total auxin present in corn kernels is extremely small, but immediately after pollination the auxin content increases very sharply; a peak is reached in from one to three weeks, at which time the kernels contain the equivalent of 0.3 to 0.5 mg. indoleacetic acid per gram dry weight. From the peak to the dormant stage, there is a marked drop in auxin content which is characteristic of all the varieties studied.

No relationship was apparent between vegetative vigor of hybrids and the amount of auxin stored in the kernels produced by them.

No fixed relationship exists between the amounts of auxin precursor and free auxin, except that the precursor always constitutes the major portion of the total obtainable at any time.

Corn kernels with sugary endosperm are consist-

ently higher in total auxin than those with starchy endosperm.

Exploratory experiments with immature kernels of diploid and tetraploid corn also failed to reveal

any evidence of a relationship between polyploidy and the amount of auxin stored in kernels.

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AN ACCOUNT OF THE SPECIES OF POLYSIPHONIA ON THE PACIFIC COAST OF NORTH AMERICA. I. OLIGOSIPHONIA¹

George J. Hollenberg

THE NAME *Polysiphonia* was first proposed by Greville (1824). *Hutchinsia* had been previously proposed for this group of plants by C. A. Agardh (1817), but proved inadmissible because of prior application of this name by Robert Brown to a group of cruciferous plants. At least fourteen other names have been employed at one time or another to designate this group, as listed by De Toni (1903). When first proposed, *Polysiphonia* included practically all of the evidently segmented plants now placed in the Rhodomelaceae. As finally delimited by Falkenberg (1901), *Polysiphonia* includes those radially symmetrical members of the Rhodomelaceae in which (1) at least the ultimate branches are evidently polysiphonous; (2) most of the branches arise exogenously by a more or less diagonal division of subapical cells before these have cut off pericentral cells; (3) all branches are essentially similar and indeterminate; and (4) only one tetrasporangium is borne normally in each segment. Erect branches arise mostly as assurgent extensions of prostrate branches or exogenously, but they may also arise endogenously from the central cells after the formation of pericentral cells.

Endogenous branches in *Polysiphonia* and closely related genera may usually be distinguished from exogenous branches, not only by their manner of origin, but also by the nature of the basal segment of the branch at the point of insertion. In exogenous branches there is usually a reduced number of pericentral cells in the basal segment. This is rarely if ever characteristic of endogenous branches. Furthermore, basal segments of exogenous branches are usually considerably shorter than corresponding segments in the parent branch and also considerably

shorter than more distant segments of the same branch.

Falkenberg (1901, p. 55) describes two types of endogenous origin of branches in the Rhodomelaceae. Normal endogenous branches arise at certain definite positions and in regular sequence in relation to the shoot apex. Adventitious branches arise endogenously at almost any position and do not bear a regular sequence relationship to the origin of other branches. In *Polysiphonia* all branches are adventitious since they do not arise in constant positions in regular sequence with respect to the segments. Although for the most part the branches are not endogenous, in several specimens of *P. hemisphaerica* Aresch. from the Baltic, which were examined, all branches seemed to be endogenous.

It seems also desirable to distinguish two types of exogenous branch. These may be designated as normally exogenous branches, which arise directly from branch primordia cut off from the subapical cells, and cicatrigenous branches, which arise from scar-cells. The scar-cells are persistent basal cells of trichoblasts, commonly left when the trichoblasts or hairs are shed. Since in *Polysiphonia* all trichoblasts arise exogenously, all cicatrigenous branches are indirectly exogenous. In *Polysiphonia* most of the so-called accessory branches are cicatrigenous, but the term accessory seems to be used somewhat loosely in this connection.

Falkenberg distinguishes two morphological or developmental types of branch in the Rhodomelaceae. Determinate branches, which do not ordinarily give rise to further branches, he designates as "Kurztriebe," whereas indeterminate branches of potentially unlimited growth are designated as "Langtriebe." In *Polysiphonia* all branches are at least

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theoretically indeterminate, although in certain species, and especially in certain varieties of *P. pacifica* of this paper, the ultimate branchlets are commonly more or less determinate.

As has been shown by Falkenberg (1901) and other investigators, the number of pericentral cells is usually quite constant if there are only four, but the number becomes in general more variable as the number of pericentral cells is increased. Tetrasporic branches usually appear to have one or two more pericentral cells than have the vegetative branches of the same plant, due to the formation of two cover cells by division of the fruiting pericentral cell.

With respect to the sequence of origin of pericentral cells in *Polysiphonia*, reference may be made to the work of Falkenberg (1901) and Rosenberg (1933). No data in regard to this question are available in the case of the species treated in this paper.

Trichoblasts or hairs are present in most species of *Polysiphonia*. In a few species trichoblasts are entirely wanting. In other species they are exceedingly rare or seasonal in their occurrence, or occur only in connection with sexual reproductive structures. As pointed out by Falkenberg (1901), trichoblasts always arise exogenously. In most cases they are soon deciduous. The basal cell is usually very short and persists as an inconspicuous scar-cell at the node between the pericentral cells. In certain species some of the trichoblasts, especially those in whose axils branches arise, do not leave scar-cells. Under circumstances as yet poorly understood, trichoblast primordia may remain undeveloped and form persistent scar-cells directly. As has been already pointed out, the scar-cells may sometimes give rise to cicatrigenous branches, but most of them do not.

Trichoblasts usually arise in very definite positions, which have been shown by Falkenberg (1901) to be of very great taxonomic importance. There seem to be no known cases in which more than one trichoblast has arisen on a given segment. Most commonly a single segment intervenes between successive trichoblasts, but in some species two or more segments occur between successive trichoblasts. The trichoblasts are usually arranged in a spiral line running in a counter-clockwise direction toward the tip of the branch, when the branch is viewed from the apex. Accordingly, Rosenberg (1933) and other investigators, who have studied the origin of branches and trichoblasts in *Polysiphonia*, speak of the trichoblasts as usually spiralling to the left. Plants are rarely found in which the direction of the spiral is reversed. The divergence of the trichoblasts in the spiral is relatively constant and usually bears some relation to the number of pericentral cells.

In most of the species of *Polysiphonia* found on the Pacific coast of North America the position of the trichoblasts is a very constant feature, but in at least one local species they are very irregularly placed, as they are in a number of European species. Even in such cases, however, there is often a distinct

tendency for the trichoblasts to be arranged in definite positions.

Exogenous branches, arising subapically, almost invariably appear in positions comparable to those in which the trichoblasts arise and in the same spiral line, so that trichoblasts and branches are homologous structures with respect to origin. In some cases the entire primordium becomes the branch, but in other species the primordium divides and one fork, usually the outer one, develops into a trichoblast and the other one develops into the branch. Hence various investigators speak of branches as originating in connection with trichoblasts or not in connection with trichoblasts (replacing trichoblasts). Trichoblasts in whose axils branches arise are sometimes spoken of as "leaves."

In certain species of *Polysiphonia* the pericentral cells are more or less obscured by the development of an outer layer of cortical cells. The cortical filaments arise from small cells cut off in most cases from the corners of the pericentral cells.

As has been shown by Falkenberg (1901) and confirmed by later studies, the vegetative features, briefly discussed in the preceding paragraphs, are much more important taxonomically than are the reproductive features. The latter exhibit for the most part a monotonous uniformity. Accordingly an extended discussion of the reproductive structures of *Polysiphonia* is neither desirable nor necessary for the present purpose. Such differences as do occur will be mentioned in connection with the different species in so far as reproductive structures are known.

A great deal of synonymy has accumulated in the genus *Polysiphonia*, especially in the tetrasiphonous members of the genus. Much of this synonymy could probably have been avoided if more attention had been given to critical structural features, which have for the most part been pointed out by Falkenberg (1901). Especially important are the positions of trichoblasts and scar-cells; origin of branches, whether endogenous or exogenous, whether entirely replacing trichoblasts or arising in connection with them; and the nature of the antheridial branches and their relation to trichoblasts. Another feature of considerable taxonomic significance which needs more attention is the nature and origin of attachment organs. A monographic treatment of the genus is greatly needed, but can hardly be undertaken in the present state of international relations.

Twenty-one species of *Polysiphonia* are known to the writer from the Pacific coast of North America. One of these, *P. Brodiaei* Grev., is practically indistinguishable from the European plant of that name and may prove to be an introduced species. *P. pacifica* n. sp. is closely similar to *P. urceolata* (Lightf.) Grev. from Europe and the Atlantic coast of North America. *P. curta* Montague and *P. paniculata* Montagne and possibly *P. pacifica* are found also on the Pacific coast of South America. Although not at present evident, it seems probable that further study may show some definite affinities between the

species on the two sides of the Pacific basin. Within the range of the study, *P. paniculata* and varieties of a species which has been heretofore designated as *P. sancti-petri* Collins, as well as varieties of *P. pacifica*, are found along the entire Pacific coast of North America. *P. Macounii* n. sp. is known only from the region of Vancouver Island, and *P. pungens* n. sp. only from Vancouver Island to the southern Alaskan coast. *P. Brodiaei* and *P. Snyderae* Kylin occur from Washington to southern California, and *P. flaccidissima* n. sp. occurs from central to southern California. The remaining thirteen species are known only south of Point Conception on the coast of California. Two species are so far known only from the Gulf of California.

Along the California coast there seems to be no marked seasonal fluctuation in the occurrence of the various species of *Polysiphonia*, except in the case of *P. flaccidissima*, which seems to occur in abundance in characteristic locations only during the fall and winter months. There is likewise little or no evidence of seasonal variation along the coast of Oregon and Washington, but insufficient collections are available for conclusive statements. Collections from Alaska are of course very inadequate.

During the progress of the study all specimens from the region were examined which are to be found in the following herbaria (designated in the treatment of species by the accompanying symbols): University of California (C); Farlow Herbarium (F); Marshall Field Museum of Natural History (Fi); Hopkins Marine Station (Hop.); New York Botanical Garden (N.Y.); University of Washington (W); and the herbarium of the writer (H). In addition the writer has been privileged to examine specimens collected by Dr. Francis Drouet and Mr. Donald Richards along the coast of Sonora, Mexico, in 1939, by Mr. E. Yale Dawson in the Gulf of California in 1940, and by Dr. W. R. Taylor off the west coast of Mexico during the Hancock expedition of 1934. The writer is grateful for the opportunity of examining material from all of these sources, and is especially indebted to the late Dr. N. L. Gardner of the University of California whose numerous collections along the entire Pacific coast of North America have so greatly facilitated this study. Of the many specimens examined only a few representative specimens other than the types will, in the case of most species, be cited. Specimens in the above mentioned herbaria have been fully annotated for the most part and citation in this paper seems unnecessary.

The tetrasiphonous species will be treated in part I of this paper, and the species with five or more pericentral cells will be taken up in part II to appear at a later date.

Key to the species of Polysiphonia on the Pacific Coast of North America

1. Plants with 4 pericentral cells..... 2
1. Plants with more than 4 pericentral cells (Part II of this paper)12
2. Trichoblasts and scar-cells wanting or very rare; tetrasporangia in straight series..... 3
2. Trichoblasts or scar-cells frequent to abundant.. 4
3. Ultimate branchlets sharply pointed and more or less determinate.....*P. pungens* n. sp.
3. Ultimate branchlets not sharply pointed, although frequently more or less determinate.
P. pacifica n. sp.
4. Trichoblasts and scar-cells infrequent and irregularly inserted.....*P. sonorensis* n. sp.
4. Trichoblasts and scar-cells frequent and in regular positions 5
5. Trichoblasts and branches alternating in spiral succession 2-3 segments apart and with $\frac{1}{4}$ divergence.
P. decussata n. sp.
5. A trichoblast, scar-cell, or a branch occurring on every segment 6
6. Plants with longitudinal cortical filaments on older parts.....*P. Macounii* n. sp.
6. Plants without cortical filaments..... 7
7. Plants minute tufted epiphytes mostly under 1 cm. high, with endophytic rhizoids composed of two cells.....*P. minutissima* n. sp.
7. Plants larger, rhizoids unicellular..... 8
8. Segments in main axes rarely longer than broad.. 9
8. Segments in main axes commonly longer than broad10
9. Main axes very prominent and straight; plants tufted, mostly epiphytic.....*P. acuminata* Gard.
9. Main axes less prominent, plants forming low mats on rocks.....*P. simplex* n. sp.
10. Branching dichotomous throughout; plants with discoid attachment.....*P. Masonii* S. & G.
10. Branching not dichotomous throughout; attachment usually not discoid.....11
11. One fork of branch primordia forming a trichoblast, branches arising accordingly in the axils of trichoblasts.....*P. flaccidissima* n. sp.
11. Branch primordia not forming trichoblasts but entirely replacing them.....*P. Snyderae* Kylin

POLYSIPHONIA *pungens* sp. nov. (fig. 1, 10).—Plantae 10–15 cm. altae, profuse ramosae, cum ramis fere laxis praecipue deorsum; axibus principibus 180–260 μ crassis, cum segmentis 8–15 diametros longis; subultimis ramis virgatis et in segmentis 2–4 ramula breviora et recta et determinata et acute pungentia undique ferentibus; ramulis apicem ramorum parce aut non excedentibus; cellulis pericentralibus 4, omnino ecorticatis; trichoblastis et cicatricellulis deficientibus; tetrasporangiis in seriebus rectis in ramulis ultimis; cystocarpis et ramis antheridialibus ignotis; colore rubro aut fusco-rubro.

Plants 10–15 cm. high, profusely branched, with branching mostly lax, especially below; main axes 180–260 μ in diameter, composed of segments 8–15 diameters long; subultimate branches virgate, bearing at intervals of 2–4 segments relatively short straight determinate and sharply pointed branchlets on all sides; branchlets not at all or scarcely exceeding the branch tips; pericentral cells 4, without cortication; trichoblasts and scar-cells wanting; tetrasporangia in straight series in the ultimate branchlets; cystocarps and antheridia unknown; plants red or brownish purple, known only from the vicinity of Vancouver Island, British Columbia and from Alaska.

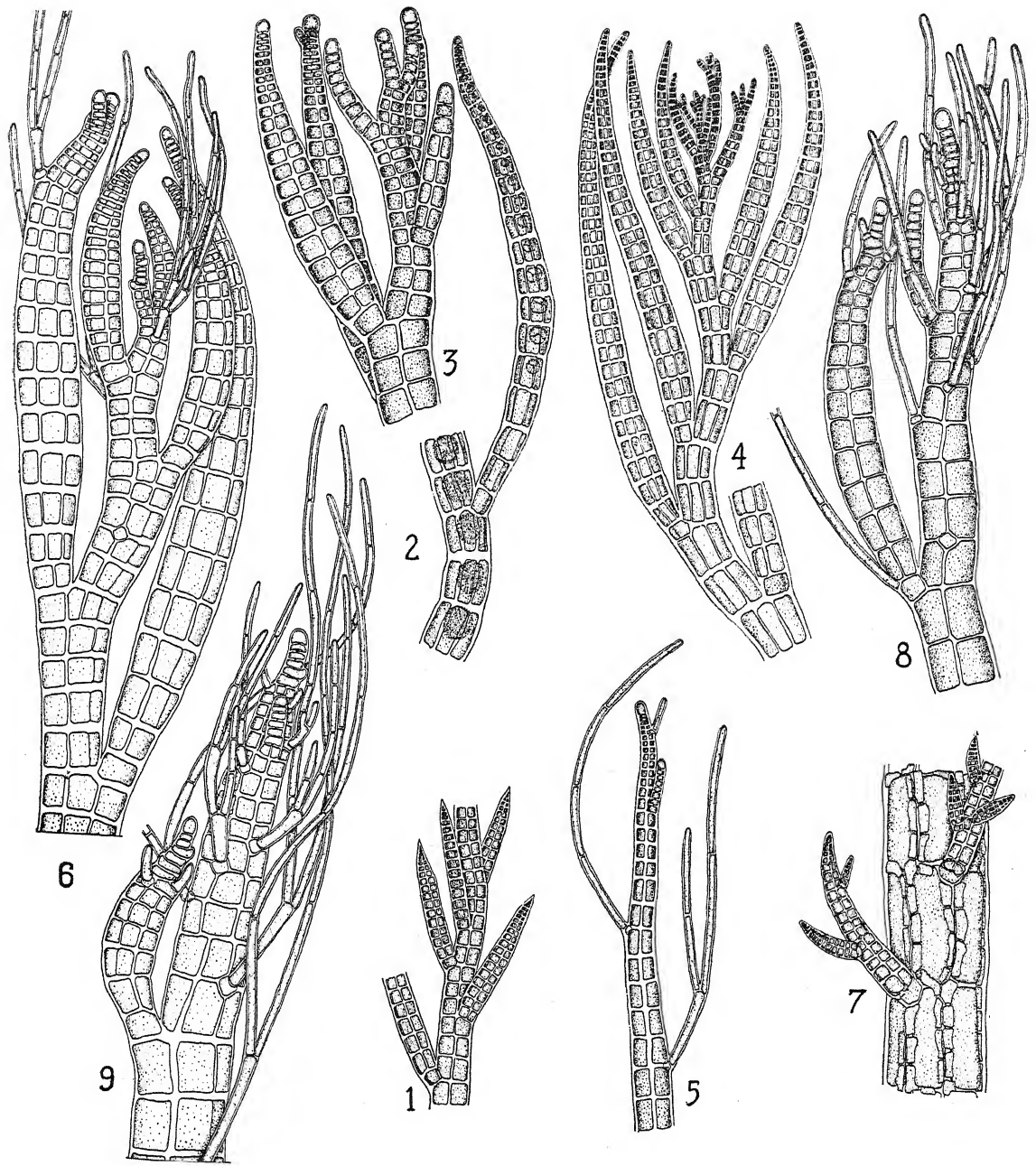


Fig. 1-9. All drawings were made with the aid of a camera lucida.—Fig. 1. Portion of a branch of *P. pungens* n. sp. $\times 85$.—Fig. 2. Tetrasporic branch of *P. pacifica* n. sp. $\times 80$.—Fig. 3. Tip of a branch of *P. pacifica* n. sp. $\times 360$.—Fig. 4. Branch tip of *P. pacifica* var. *disticha* n. var. $\times 85$.—Fig. 5. Branch tip of *P. sonorensis* n. sp. $\times 70$.—Fig. 6. Branch tip of *P. decussata* n. sp. $\times 360$.—Fig. 7. Portion of a larger branch of *P. Macounii* n. sp. $\times 17$.—Fig. 8. Branch tip of *P. flaccidissima* n. sp. $\times 325$.—Fig. 9. Branch tip of *P. Snyderae* Kylin. $\times 360$.

Type no. 314925 in the herbarium of the University of California, collected by R. B. Wylie on Valenar Rock, Gravina Island, lat. $55^{\circ} 26' N.$, Alaska, May, 1913. Several additional specimens from British Columbia were collected by John Macoun, of the Geological Survey of Canada about 1909, or perhaps a few years previous to this time: Macoun 34, without date or locality (F); Macoun 93, Vancouver Is.,

without date (C); Macoun 136, Qualicum, Vancouver Is., without date (N.Y.).

This species is very close to *P. pacifica* of this paper, especially to those varieties with determinate branchlets. It differs in the virgate ultimate branches and the sharply pointed branchlets which they bear. The color is usually more red and not dark brownish or black as is common on *P. pacifica* var. *determinata*.

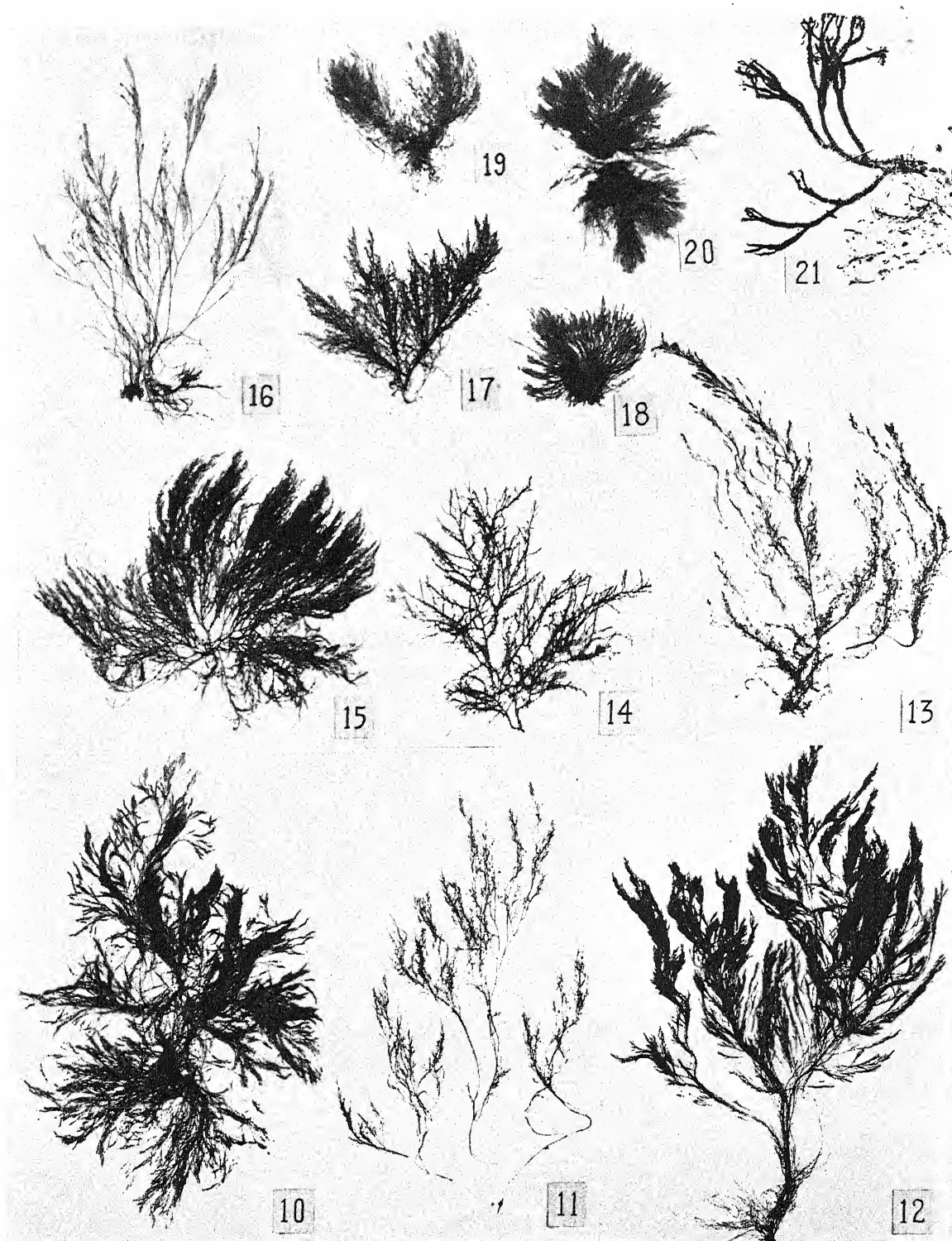


Fig. 10-21.—Fig. 10. Type specimen of *P. pungens* n. sp. $\times 0.4$.—Fig. 11. Type specimen of *P. pacifica* var. *gracilis* n. var. $\times 0.5$.—Fig. 12. Type specimen of *P. pacifica* n. sp. $\times 0.5$.—Fig. 13. A slender form of *P. pacifica*. $\times 0.5$.—Fig. 14. Type specimen of *P. Macounii* n. sp. $\times 0.5$.—Fig. 15. Type specimen of *P. Snyderae* var. *heteromorpha* n. var. $\times 0.5$.—Fig. 16. Type specimen of *P. pacifica* var. *distans* n. var. $\times 0.5$.—Fig. 17. Type specimen of *P. pacifica* var. *disticha* n. var. $\times 0.5$.—Fig. 18. Type specimen of *P. simplex* n. sp. $\times 0.5$.—Fig. 19. Type specimen of *P. flaccidissima* var. *Smithii* n. var. $\times 0.5$.—Fig. 20. Type specimen of *P. sonorensis* n. sp. $\times 0.5$.—Fig. 21. Photomicrograph

POLYSIPHONIA pacifica sp. nov. (fig. 2, 3, 12, 13). —Plantae medio-rubrae aut fusco-rubrae vel parce nigrae et molles aut firmae, cum filamentis repentibus inconspicuis, per rhizoidia unicellularia in intervallis irregularibus affixae, unum solum per segmentum, ex centro inferiorum cellularum pericentralium ut ex-crescentia orta, a quibus muris transversis non abscissa sunt; ramis erectis fere endogene ortis, (2) 10–20–(25) cm. altis, laxe ramosis praecipue infra, fere cum nudis regionibus longioribus; ramis alternantibus, interdum aliquanto distichis, cum axibus primis distinctis aut indistinctis et 60–200–(300) μ crassis; ramis multorum ordinum; ramulis fere apicem crescentem haud excedentibus; fere cum 3–5 segmentis inter ramos succedentes; cellulis pericentralibus 4, omnino ecorticatis; segmentis ramorum primorum fere 6–10 diametros longis, multo brevioribus in ramulis ultimis, qui fere apice attenuati sunt; trichoblastis et cicatricellulis rarissimis; tetrasporangiis 60–70–(100) μ crassis, in seriebus longis et rectis in ramulis ultimis, tripartite divis; cystocarpis ovoido-globosis, leviter vel manifeste urceolatis, brevius pedicellatis, 200–500 μ crassis; cylindratis ramis antheridialibus cum breve apice sterile unius aut plurium cellularum, ab toto ramo fructifero ortis; plantae fere saxis adhaerentes.

Plants medium to dark red or nearly black, adhering well or poorly to paper, with inconspicuous creeping filaments attached by unicellular rhizoids arising at irregular intervals, never more than one per segment, as outgrowths from the center of the lower pericentral cells, from which they are not cut off by cross walls; erect branches arising mostly endogenously, 10–20–(25) cm. high, mostly laxly branched, especially below, with relatively long naked regions; branching alternate, sometimes somewhat distichous, with main axes distinct or indistinct, 100–200–(300) μ in diam.; branches of many orders, typically not exceeding the growing tip, with (2)–4–5—many segments between successive branches; pericentral cells 4 in vegetative branches, totally ecorticate, the segments in the main branches (4)–6–10–(20) diam. long, well separated by clear septa and much shorter in the ultimate branchlets, which are commonly considerably attenuate at the tip but not pungent; trichoblasts and scar-cells exceedingly rare; tetrasporangia 60–70–(100) μ diam., in long straight series in the ultimate branchlets, tripartitely divided; cystocarps slightly to conspicuously urceolate, briefly pedicellate, 200–500–(675) μ in diam.; antheridial branches cylindrical, with a short sterile tip of one to several cells, formed from the entire fruiting branch; plants growing on rocks, pilings, etc., in the lower littoral and sublittoral zone from Alaska to Lower California.

A specimen (fig. 12) in the herbarium of the New York Botanical Garden has been selected as the type. It was collected by Dr. C. L. Anderson at Santa Cruz but bears no date. It is tetrasporic. A more delicate representative of the species is shown in figure 13.

of a portion of a male plant from the type material of *P. minutissima* n. sp. $\times 15$, showing the basal tuft of rhizoids in the lower right hand corner.

This plant was collected by N. L. Gardner, 2750, from stones and logs, at Empire, Coos Bay, Oregon. *P. pacifica* is a very variable species, as is *P. urceolata* (Lightf.) Grev., to which it is closely related. The latter plant is common in Europe and along the Atlantic coast of North America. In both plants the antheridial branch arises from the entire trichoblast primordium. The sterile tip is generally much shorter, however, in the Pacific coast plant. In both plants the rhizoids arise as extensions from the middle of the lower pericentral cells and are not cut off by cross walls from the pericentral cells. The Pacific Coast plant is in general somewhat more robust and a much more variable plant. Furthermore, trichoblasts and scar-cells are much more rare in the case of the Pacific Coast plant. These structures are reputed to be rare in the case of *P. urceolata*, but the writer has been able to find either trichoblasts or scar-cells in nearly every specimen of *P. urceolata* which was examined from the Atlantic coast of North America and in many of those from Europe. In over a hundred specimens of *P. pacifica* which were examined, representing collections during all seasons of the year, only one instance of either trichoblasts or scar-cells was observed except for occasional trichoblasts on cystocarps and scar-cells left by deciduous antheridial branches.

Among the specimens of *Polysiphonia* from Peru, which M. A. Howe (1914) identified as *P. abscissa* Hook. and Harv., several seem identical with typical specimens of *P. pacifica*, but as Howe states, several entities are probably represented in the Peruvian material under this name, and the writer has been unable to obtain authentic material of *P. abscissa* from the Cape Horn region from which the type came.

Key to the varieties of *P. pacifica*

1. Plants small and delicate, mostly under 4 cm. high.
P. pacifica var. *delicatula* n. var.
1. Plants larger and more robust, commonly 10–25 cm. high 2
2. Ultimate branchlets relatively short, rigid and more or less determinate..... 3
2. Ultimate branchlets indeterminate..... 5
3. Ultimate branchlets more or less distichous, main axes usually covered with branchlets throughout..... *P. pacifica* var. *disticha* n. var.
3. Ultimate branchlets not noticeably distichous, main axes usually with long naked portions below 4
4. Branching usually dense, main axes macroscopically indistinct above and commonly exceeded by the uppermost branchlets.
P. pacifica var. *determinata* n. var.
4. Branching lax to very lax, main axes distinct to the tips, which are usually not exceeded by the upper branchlets..... *P. pacifica* var. *gracilis* n. var.
5. Branches mostly delicate and soft, abundantly branched; color red..... *P. pacifica* n. sp.
5. Branches coarse to very coarse, up to 300 μ in diameter, distant; color usually very dark.

P. pacifica var. *distans* n. var.

P. pacifica var. **delicatula** var. nov.—Plantae rubrae, 0.5–2.0 cm. altae; axibus principibus 60–110 μ crassis, cum segmentis 1.5–2–(3) diametros longis; ramis omnibus indeterminatis, cum apicibus brevibus et fere obtusis; apicibus frequenter aliquanto forcipatis; trichoblastis raris; tetrasporangiis 50–60 μ crassis in seriebus rectis; cystocarpis aperte sine trichoblastis; ramis antheridialibus tenuibus, 140–200 \times 30–40 μ crassis, in pediculis brevissimis, cicatricellulas persistentas relinquentibus; plantae ad saxa et sublicas, fere in aqua profunda aut placida, affixae.

Plants red, 0.5–2.0 cm. high, rarely higher, main branches 60–110 μ diam., of segments 1.5–2–(3) diameters long; branches all indeterminate short and mostly blunt; branch apices often somewhat forcipate; trichoblasts wanting or exceedingly rare; tetrasporangia 50–60 μ in diam., in straight series; cystocarps seemingly without hairs; antheridial branches slender, 140–200 \times 30–40 μ , on very short pedicels, leaving persistent scar-cells when shed; plants occurring from central California to the Gulf of California, Mexico, on pilings and rocks in deep or sheltered water.

Type: Hollenberg 2888, collected from low tide level on pilings of the municipal wharf at Monterey, California, July 20, 1939, male and cystocarpic (H.). This plant has more rigid and more forcipate tips than have plants from the lagoons of southern California and from the Gulf of California. The scar-cells left by deciduous antheridial branches indicate that the antheridial branches are homologous with trichoblasts and not with branches of the thallus as Falkenberg (1901) concluded in the case of the similar antheridial branches of *P. urceolata*.

P. pacifica var. **disticha** var. nov. (fig. 4, 17).—Plantae 3–7 cm. altae, cum structura et ramis fructiferentibus ut in specie, sed plantis fere parvioribus, cum ultimis ramulis determinatis, primum incurvatis, demum rectis aut recurvatis, cum apicibus acutis, spiraliter alterne aut vulgo in alternis geminis, in intervallis 2–3 segmentorum, per totam longitudinem omnium ramorum ortis, vulgo partim distichis aut aperte distichis fere propter axem modice torsum.

Plants 3–7 cm. high, with chief structural features and reproduction as in the species, but plants mostly smaller with ultimate branchlets determinate, at first incurved, and finally straight or strongly incurved, with acute tips, spirally arranged in an alternate manner or commonly in alternating pairs every 2–3 segments throughout the length of all branches, commonly partly distichous or apparently distichous as a result largely of twisting of the axis; plants occurring mostly in the upper littoral zone in exposed places, central California to Vancouver Island, British Columbia, and probably extending northward.

Type: no. 276091 (fig. 17) in the herbarium of the University of California, collected by N. L. Gardner, 3888a, on the ocean side and three miles south of Cape Flattery, Washington, June, 1917. Other specimens examined include: N. L. Gardner 4405, from a stone wall above high tide level, "Fort Point," San

Francisco, California, Feb. 1919 (C., H.); and N. L. Gardner 3850, from the type locality, April, 1927 (C.). This variety has more or less determinate branchlets as in var. *determinata*, in which the branchlets are likewise somewhat distichous at times, but var. *disticha* is usually much smaller and its main axes are clothed with more or less determinate branchlets below as well as above.

P. pacifica var. **determinata** var. nov.—Plantae fere 10–15 cm. altae, deorsum laxae, cum axibus principibus 170–340 μ crassis, et segmentis deorsum 6–8 diametros longis, sursum brevioribus; ultimis ramulis crassioribus, aliquanto determinatis et plerumque cristas densas et aliquanto pencillatas formantibus, plerumque apicem crescentem aperte excedentibus, fere non distichis; trichoblastis solum in exemplis raris visis; tetrasporangiis et ramis antheridialibus ut in specie; cystocarpis magnis et aliquanto urceolatis; plantae fusco-rubrae.

Plants mostly 10–15 cm. high, lax below, with main axes 170–340 μ in diameter and segments 6–8 diameters long below, and shorter above: ultimate branchlets relatively coarse, more or less determinate and often forming more or less dense pencillate tufts, often extending well beyond the growing point of the branch, and mostly not distichous; trichoblasts wanting except for very rare instances; tetrasporangia and antheridial branches as in the species; cystocarps relatively large and more or less urceolate; plants dark brownish red to nearly black when dry, adhering only moderately well to paper from which they are readily loosened on moistening, occurring from central California to Alaska.

Type: N. L. Gardner 3827, on rocks, low littoral zone in cove beyond the first point south of Pebble Beach, Monterey County, California, May 1916 (C. 276272); Phyc. Bor. Amer. 1697, at least in part, John Macoun, Vancouver Is., British Columbia.

Many additional specimens of this common variety are to be found in the various herbaria mentioned. Similarities between this and the preceding variety have already been mentioned in connection with the discussion of that variety. In no. 3888 (C., H.) collected by N. L. Gardner from the upper littoral tide pools along the open coast 3 miles south of Cape Flattery, Washington, frequent forked hairs were present toward the branch tips, and occasional scar-cells were present. They were not in any constant positions, however, and the tetrasporangia were in straight series as in the species.

P. pacifica var. **gracilis** var. nov. (fig. 11).—Plantae obscure fusco-rubrae, 10–20 cm. altae, laxiores ramosae; ramis ordinum plurium, cum axibus principibus distinctioribus, vulgo deorsum nudis, sursum cum 1-multis subramulis; structura et tetrasporangiis ut in specie; cystocarpis et antheridiis ignotis.

Plants dull brownish red, 10–20 cm. high, very laxly branched in most cases; branches of several orders, with leading axes very distinct and commonly

naked below, above with slender lateral branchlets, which are more or less determinate and mostly simple or with 1-several short ramuli toward their tips; finer structural details and tetrasporangia as in the species, cystocarps and antheridia unknown; plants known only from the vicinity of San Juan and Orcas Islands along the coast of Washington.

Type: N. L. Gardner 2313, dredged in Deer Harbor, Orcas Island, Washington, July, 1910 (No. 273756 in the Univ. of Calif. herbarium). A single additional collection is from the same general locality, by J. S. Martin 33, extreme low littoral, exposed to swift currents, Twin Rock, 2 miles east of Friday Harbor, San Juan Island, Washington, Aug. 1940 (W.). This variety is distinguished by its slender laxly branched habit and determinate, mostly unbranched branchlets. There are many segments between successive branches in the lower parts, but mostly four between the ultimate or subultimate laterals. Segments in the main branches are many diameters long below and mostly 2 diameters long above. Lateral branchlets which in turn bear short ramuli usually have 20-30 segments between the base of the branchlet and the first ramulus.

P. pacifica var. *distans* var. nov. (fig. 16).—Plantae crassulae sed flexiles, fusco-rubrae, 10-18 cm. altae, cum axibus principibus ad 300 μ crassis et segmentis 2-4-(8) diametros longis; ramis distantibus praecipue deorsum, cristas pencillatas non formantibus; ramulis juvenibus saepe valide incurvatis; cystocarpis urceolatis, 550-675 μ crassis, cum labro ostioli crasso et fere quadralobato; tetrasporangiis in seriebus brevibus ad apicem ramulorum breviorum et ultimorum; plantis antheridialibus ignotis.

Plants coarse but limber, dark brownish-red, drying darker, 10-18 cm. high, with main axes up to 300 μ in diam., of segments 2-4-(8) diameters long; branching distant especially below; branches not forming pencillate tufts; young branchlets often strongly incurved; tetrasporangia in short series near the tips of short ultimate branchlets; cystocarps urceolate, 550-675 μ in diam., with thick mostly four-lobed ostiolar rim; antheridial plants unknown; plants adhering only moderately well to paper, occurring from British Columbia to central California.

Type: Hollenberg 2722, cystocarpic, afloat at the south end of Carmel Beach, Monterey County, California, June 22, 1939 (H.), (Isotype, Hop.). Three additional collections may be mentioned; Port Renfrew, British Columbia, Misses E. Butler and J. Polley 112, without date but probably about 1900, and distributed by W. A. Setchell as *P. senticulosa* Harv. (C.); Vancouver Island, B. C., without date, Robert Connell, tetrasporic (C.); Santa Cruz, C. L. Anderson without date, with some of the characteristics of *P. pacifica* var. *determinata* (N. Y.). The coarse laxly branched filaments, very large cystocarps, and tetrasporangia in short series seem the distinctive features of this variety.

POLYSIPHONIA sonorensis sp. nov. (fig. 5, 20).—Plantae 4-5 cm. altae ex ramis prostratis et repentibus; ramis prostratis 120-175 μ crassis, per rhizoidia unicellularia in intervallis irregularibus affixis; rhizoidiis uno solo per segmentum, ex centro inferiorum cellularum pericentralium excrecentibus, per muros transversos non abscissis; erectis ramis ex ramis prostratis fere endogene aut interdum exogene ortis, 70-100 μ crassis, cum segmentis fere 1.5-2 diametros longis, aliquanto dense ramosis, cum ramis lateralibus exogene fere in angulis acutis orientibus in intervallis irregularibus fere 10-20 segmentis separatis; ramis principibus indistinctis; ramulis ultimis tenuibus, 30-35 μ crassis, ad fundamentum modice attenuatis, ex segmentis fere unum diametrum longis aut brevioribus; cellulis pericentralibus 4, ecorticatis; trichoblastis tenuibus et numerosis ad 1 mm. longis, in intervallis irregularibus, cum 1-2 furcis, deciduis, frequenter cicatricellulas persistentes relinquentibus; ramis cum trichoblastis in origine non conjunctis; ramis fructiferentibus ignotis; plantis colore similibus lateri.

Plants forming dense tufts of a terra cotta color, 4-5 cm. high from prostrate creeping filaments; prostrate filaments 120-175 μ diam., attached by unicellular rhizoids which arise as outgrowths of the center of the pericentral cells, from which they are not cut off by cross walls; erect branches arising mostly endogenously or sometimes exogenously from the prostrate branches, 70-100 μ in diam., their segments mostly 1.5-2.0 diam. long; branching dense, the lateral branches arising exogenously at irregular intervals (4)-10-20 or more segments apart, mostly at an acute angle with the main axis, which is usually indistinct; ultimate branchlets slender, 30-35 μ in diam., slightly narrowed at the base, of segments mostly 1 diam. long or shorter; pericentral cells 4, not corticated; trichoblasts numerous, delicate, arising at irregular intervals, once or twice forked, to 1 mm. long, deciduous, often leaving inconspicuous scar-cells; branches not associated with trichoblasts in origin; reproduction unknown; plants adhering moderately well to paper on drying but readily removed, known only from the Gulf of California.

The type specimen is no. 3426a collected by Francis Drouet and Donald Richards afloat along the southeastern shore of the bay of Empalme, Sonora, Mexico, Dec. 23, 1939 (Fi.). The same investigators also collected this plant as no. 3144 (Fi.) from rocks between tide marks on the northwest shore of the harbor at Guaymas, Sonora, Mexico, on Dec. 4 of the same year.

In the nature and origin of the rhizoids, the pelucid nature of the transverse septa, and general structural details this plant closely resembles *P. pacifica*. It differs from the latter in the numerous irregularly occurring trichoblasts, which are exceedingly rare in *P. pacifica*; and also in having a greater number, and a more irregular number, of segments between successive branches. The frequent trichoblasts suggest a possible affinity with *P. urceolata* (Lightf.) Grev., but in the latter species the tricho-

blasts when present seem to be more regularly arranged in a spiral with one-fourth divergence according to Rosenvinge (1924) and other investigators. Furthermore, according to Falkenberg (1901) there is a more or less constant interval of 3-4 segments between successive branches in *P. urceolata*. Finally the terra cotta color of *P. sonorensis* is seemingly distinctive. From the imperfectly known *P. subtilissima* Montagne the present species differs in color, in the narrower angle of insertion of the branches, and in the manner of branching which does not seem at all dichotomous below in *P. sonorensis* as described for *P. subtilissima*. The Pacific coast plant also has more numerous trichoblasts than have specimens of *Polysiphonia* from the coast of Mississippi identified as *P. subtilissima* by W. R. Taylor.

POLYSIPHONIA decussata sp. nov. (fig. 6).—Plantae 1-2 cm. altae, assurgentes ex fundamento prostrato et repente, 150-180 μ crasso et cum segmentis fere circiter unum diametrum longis, per rhizoidia unicellularia 160 aut etiam 800 μ longa cum apicibus lobatis affixo; rhizoidiis in brevioribus intervallis ortis, fere unum per segmentum, ab fine proximale cellularum pericentralium a quibus muro transverso non abscissa sunt; ramis erectis 150-170 μ crassis, cum segmentis 0.5-1.0-(1.5) diametros longis; ramis plurium ordinum, exogenibus aut rare cicatrigenibus, fere alternante distichis in 4-6 segmenta; ramulis ultimis forte incurvatis, saepe apices ramorum excedentibus et superarcuantibus; cellulis pericentralibus 4, circum parvum cellulam centralem, omnino ecorticate; trichoblastis simplicibus aut semel aut bis furcatis, 200-360 longis, decussate alternantibus cum ramis ita dispositis ut rami cum trichoblastis aut cicatricellulis persistentibus laevam spiram quantum divergentum faciant; trichoblastis aut cicatricellulis distichis in ramulis ultimis, fere 2 segmenta separatis; cicatricellulis saepe ex trichoblastorum primordis orientibus; tetrasporangiis 35-60 μ crassis, in seriebus aliquanto continuis in ramulis ultimis, irregularibus sed apparenter in spiris non positis; cystocarpis ovoidis, aliquanto urceolatis, breve pedicellatis 250-320 x 300-430 μ crassis, in loco rami; ramis antheridialibus 30-40 x 100-150 μ , ramum primum trichoblasti constituentibus; plantae colore fusco-russeo, saxis adhaerentes.

Plants 1-2 cm. high, assurgent from a prostrate creeping base 150-180 μ in diam. and composed of segments mostly about 1 diameter long, attached by unicellular rhizoids up to 160 or even 800 μ long with lobed tips; rhizoids arising at short intervals, seldom more than one per segment, from the proximal end of the pericentral cells from which they are not cut off by a cross wall; erect branches 150-170 μ in diam., their segments 0.5-1.0-(1.5) diam. long; branches of several orders, exogenous or rarely cicatrigenous, arising in an alternately distichous manner every 4-6 segments; ultimate branchlets strongly incurved, often exceeding and overarching the branch tip; pericentral cells 4 around a small central cell, totally ecorticate; trichoblasts simple or

once or twice forked, 200-360 μ long, decussately alternate, with the branches so arranged that they, together with the trichoblasts or persistent scar-cells, form a left hand spiral with $\frac{1}{4}$ divergence, 2-3 segments intervening between a branch and the next higher or lower trichoblast in the spiral; trichoblasts and scar-cells distichous on the ultimate branchlets, mostly 2 segments apart; scar-cells often represented by undeveloped trichoblast primordia; tetrasporangia 35-60 μ in diam., in more or less continuous series in the ultimate branchlets, shifting in position but seemingly not in a regular spiral arrangement; cystocarps ovoid, somewhat urceolate, briefly pedicellate, measuring 250-320 x 300-430 μ , replacing a branch; when young surmounted by a slender forked hair; antheridial branches 30-40 x 100-150 μ , arising as a primary branch of a trichoblast; plants deep reddish brown, forming tufts or somewhat matted on rocks or intermingled with other matted algae.

Type: Hollenberg 2649, cystocarpic, tufted, on boulders, medium low tide level, near the mouth of Topango Canyon, Los Angeles County, California, June 4, 1939 (H.). Only two other collections have been made: at Fairview Point, Laguna Beach, Orange County, California, Hollenberg 1114, tetrasporic, Nov. 1935 (H.); and at Park Point, LaJolla, San Diego County, California, in the middle littoral zone on rocks, Hollenberg 2634a, male, May, 1939 (H.).

This diminutive plant seems very distinct from other Pacific coast species, especially in the arrangement of the branches and trichoblasts. Branch tips frequently have the aspect of a *Pterosiphonia*, but it is excluded from that genus because of the trichoblasts and the nondecurent insertion of the branches and shortened basal segments of branches. The arrangement of trichoblasts and branches is very similar to that of a series of plants from the Atlantic coast of both North and South America, which have been commonly identified as *Polysiphonia subtilissima* Montagne. However, the Atlantic coast plants are much larger, up to 12 cm. high and more irregularly branched. Several plants from the coast of Mississippi, which are considered by W. R. Taylor to be more definitely identified with the type of *P. subtilissima*, were found by the writer to have little if any regularity in the insertion of the branches. They are much more laxly branched than the Pacific coast plants and young branchlets are not at all incurved.

POLYSIPHONIA Macounii sp. nov. (fig. 7, 14).—Plantae 10-12 cm. altae ex ramis repentibus et dense implexis, per rhizoidia unicellularia et numerosa affixae; axibus principibus 500-600 μ crassis, cum segmentis deorsum 1-2 diametros longis et sursum brevioribus diametro; ramis aliquot vel multorum ordinum, fere exogenibus; ultimis ramulis numerosis et fere aliquanto determinatis, brevibus et calcari similibus et obtusis; cellulis pericentralibus 4, cum corticatis filamentis sparsis, in longitudinem juncturis cellularum pericentralium axis primi extensis; trichoblastis furcatis et brevibus, una per segmentum

spiram laevam quantum divergentem facientibus, mox deciduis, cicatricellulas persistentes relinquentibus; ramis a cicatricellulis frequenter ortis; ramis trichoblastorum vicem; ramis fructiferentibus ignotis.

Plants 10–12 cm. high from densely matted creeping branches attached by numerous unicellular rhizoids; main axes 500–600 μ in diam., their segments 1–2 diam. long below, and shorter than broad above; branches of several to many orders, mostly exogenous, the ultimate branchlets abundant, short obtuse and spur-like, and mostly more or less determinate; pericentral cells 4, with a number of cortical filaments extending longitudinally along the junctures of the pericentral cells in the main axes; trichoblasts short and forked, one on each segment, arranged in a left hand spiral with $\frac{1}{4}$ divergence, soon deciduous, leaving scar-cells from which accessory cicatrigenous branches may arise in considerable numbers; branches replacing trichoblasts; reproductive structures unknown; plants known only from the vicinity of Vancouver Island, British Columbia.

A series of specimens in the herbarium of the New York Botanical Garden is considered the type collection. These specimens are to be found in a packet labeled "*P. senticulosa*" from the Collins herbarium. Fragmentary notes on portions of the original wrapper indicate that this collection is no. 105, collected by John Macoun at Qualicum, Vancouver Island, British Columbia. No date is given, but John Macoun, who was naturalist on the Geological and Natural History Survey of Canada, probably collected this material about 1909 or shortly previous to this time. The only other known specimens were likewise collected by Macoun from the same general region; John Macoun no. 106, without date or locality (C.); Amphitrite Point, Barkley Sound, west coast of Vancouver Is., Macoun 93 in part (C.); and Victoria B. C., J. Macoun, without date (N. Y.).

Kylin (1941) established the genus *Orcasia* with Harvey's *Polysiphonia senticulosa* as the type species, and Kylin's photograph (fig. 33) represents a plant strikingly similar to the specimens of *P. Macounii* from the same general region. No authentic material of *Orcasia senticulosa* (Harv.) Kylin is available for examination and Kylin does not give figures of structural details. However, Macoun's specimens show no clear distinction between determinate and indeterminate branches, and the more indeterminate branches rarely if ever arise endogenously, but are mostly exogenous as indicated by the reduced number of pericentral cells in the basal segment and the absence of evidence of disturbance of the pericentral cells adjacent to the point of origin. No young branches were found arising endogenously from the base of determinate branchlets as described for *Orcasia*, and it seems doubtful if Macoun's plants can be identified with *O. senticulosa*. Some of the smaller branchlets are cicatrigenous in origin, and some of the erect branches seem to arise endogenously from the prostrate branches as in a number of species of *Polysiphonia*. Finally the main

axes of *P. Macounii* are usually corticated along the lines of juncture of the pericentral cells, whereas neither the original description of *P. senticulosa* nor Kylin's description of *Orcasia senticulosa* indicate that the plant is corticated. Lacking data concerning the origin of branches in relation to trichoblasts and concerning reproductive structures, critical comparisons with certain somewhat similar species of *Polysiphonia* are at present impossible. In general structural features *P. Macounii* is suggestive of *P. novae-angliae* Taylor from the New England region. The latter plant, however, is much more finely branched, with branches of all orders essentially similar, and the main axes are somewhat more completely corticated. From *P. fibrillosa* Grev. the west coast plant differs in color, in the spur-like branchlets, and in the lack of a discoid attachment organ. It differs from *P. subulata* (Dulc.) J. Ag. from Europe not only in the branching habit and the extent of cortication, but in the fact that branchlets are neither pectinate nor corymbosely fastigate as described for that species.

POLYSIPHONIA minutissima sp. nov. (fig. 21).—Plantae epiphyticae pusilles et cristatae, fere minus 3 mm. altae aut ad 6 mm., a crista rhizoidiorum ortae, qui in hospitem profunde penetrant; rhizoidiis e 1–3 cellulis compositis, interdum furcatis, cum apicibus obtusis et saepe aliquanto tumentibus, ad 1 mm. longis; ramis erectis cristatis, a fundamento assurgentibus; ramis complurum ordinum, exogenibus, ramis succedentibus per nulla intervalla constantia separatis; ramis principibus indistinctis, ad 145 μ crassis, cum segmentis fere unius diametri longis aut brevioribus; cellulis pericentralibus 4, omnino ecorticatis; trichoblastis brevibus, una per segmentum, in spira laeva cum $\frac{1}{4}$ declinatione, mox deciduis, cicatricellulas persistentes relinquentibus; tetrasporangiis in seriebus brevibus et spiralibus, ad 80 μ crassis, in ramulis ultimis tumentibus; cystocarpis paulo urceolatis, parce sessilibus, 225–290 μ crassis, trichoblastorum vicem; ramis antheridialibus circa 40 x 135 μ , ut unus ramus trichoblasti ortis, sine apicibus sterilibus; plantae obscure rubrae.

Plants epiphytic, diminutive, tufted, mostly under 3 mm. high or up to 6 mm. high, from a tuft of rhizoids deeply penetrating the host; rhizoids composed of 1–3 cells, often branched, with blunt and often swollen tips, up to 1 mm. long, cut off by a cross wall from the proximal end of the pericentral cells; erect branches assurgent from a prostrate rosette-like base, giving the plant the aspect of a diminutive tumbleweed; branches of several orders, exogenous replacing trichoblasts, with no constant interval between successive branches; main axes indistinct, up to 145 μ in diam., their segments mostly 1 diam. long or less; pericentral cells 4, totally ecorticate; trichoblasts short, one per segment, arising in a left hand spiral with $\frac{1}{4}$ divergence, soon deciduous, leaving persistent scar-cells; tetrasporangia in short spiral series, up to 80 μ in diam., causing the ultimate branchlets to bulge; cystocarps slightly

urceolate and nearly sessile, 225–290 μ in diam., replacing trichoblasts; antheridial branches about 40 x 135 μ , comprising one branch of a trichoblast, without sterile tips; plants dull red in color.

Type: Hollenberg 2554, collected from the middle or lower littoral zone on the bay side of Punta Banda, Lower California, Mexico, Dec. 17, 1938, growing on *Codium fragile* (Suhr.) Hariot. (H.). The writer has also collected a plant which is slightly larger but seems to belong to this species from the north side of Catalina Island, off the coast of southern California. The latter plant is up to 6 mm. high. The diminutive size and assurgent tufted habit of a tumble weed are the most distinctive features of *P. minutissima*, together with the single tuft of deeply penetrating and often multicellular rhizoids. The branching is somewhat unilateral in the case of the outer lower branches.

POLYSIPHONIA acuminata N. L. Gardner, New Rhodophyceae VI, Univ. Calif. Publ. Bot. 14:100, 1927. Plants erect, 2–6 cm. high, tufted, epiphytic or saxicolous, attached by a penetrating tuft of rhizoids arising from the base of erect branches, or having prostrate branches of limited extent which are attached by numerous rhizoids arising 1-many per segment; rhizoids unicellular and cut off by a cross wall from the pericentral cells; central percurrent axis prominent, 300–500 μ in diam., its segments mostly shorter than broad, with numerous densely branched laterals spirally arranged, resulting in slenderly conical or attenuate frond divisions; branches replacing trichoblasts, of several orders, with 2–6 (mostly 3) segments between successive branches; pericentral cells 4, relatively large, surrounding a small central cell, totally ecorticate; trichoblasts abundant 2–4 times forked, one per segment except when totally replaced by a branch, arising in a left hand spiral with $\frac{1}{4}$ divergence, soon deciduous, leaving scar-cells, the first trichoblast on a branch occurring 2–5 segments from the base of the branch, tetrasporangia 50–70 μ in diam., spirally arranged in swollen terminal branchlets; cystocarps numerous, nearly sessile, subspherical, 250–300 μ in diam.; antheridial branches measuring 100–150 x 30–50 μ , without sterile tip or with tip of 1–2 short sterile cells, arising as a primary fork of a trichoblast; plants medium to dark reddish brown, moderately adherent, growing in the upper littoral zone, chiefly in southern California.

The type material was collected by N. L. Gardner (no. 1968) at White's Point near San Pedro, Los Angeles County, California, June, 1908 (C.). It was distributed as Phyc. Bor.-Amer. no. 1599 under the name *Streblocladia camptoclada* (Mont.) Falk. Seventeen additional collections are available for study. Most of these were collected by the writer along the coast of southern California, including Catalina Island. One collection was made by N. L. Gardner in the Monterey region. The saxicolous specimens usually dry nearly black and have less distinct main axis, as compared with the epiphytic specimens. Usually the main axes are very straight and promi-

nent and the specimens are somewhat more delicate and a much lighter reddish or brownish color. The short segments in the main axes and the distinctness and straightness of these axes are in general the features which distinguish this species from *P. Snyderae* and *P. simplex* to which it is probably most closely related.

POLYSIPHONIA simplex sp. nov. (fig. 18).—Plantae saxicolae, saepe mattas densas aliquanto latas formantes, cum filamentis fundamentalibus repentibus et implicatis, 250–360 μ crassis, segmentis fere brevioribus uno diametro longis, per rhizoidia unicellularia a finibus proximalibus cellularum pericentralium abscissa et in discis lobatis terminata; filamentis prostratis ramos erectos 1–3–(7) cm. altos et 160–250 μ crassos cum axibus principibus distinctis et parce furcatis in modo exogene et assurgente producentibus; ramis exogenibus, in lateribus omnibus ortis; trichoblastis semel terque furcatis, in segmento una in collocazione spirale cum $\frac{1}{4}$ declinationibus ortis, mox deciduis, cicatricellulas persistentes relinquentibus; cum ramis trichoblastorum vicem, trichoblastis in origine non conjunctis; tetrasporangiis ad 70 μ crassis, in apicibus ramulorum aliquanto tumen-torum spiralliter dispositis; cystocarpis ovoideo-globosis, fere sessilibus, 300–350 μ crassis; ramis antheridialibus 100–170 x 35–40 μ crassis, furcis primis trichoblastorum ortis, sine apicibus sterilibus; plantae fuscae vel obscura-fuscae.

Plants saxicolous, forming dense mats often of considerable extent, with creeping tangled basal filaments 250–360 μ in diam., composed of segments mostly shorter than broad, attached by unicellular rhizoids with lobed tips, which are cut off from the proximal end of the pericentral cells by a curving wall; prostrate filaments giving rise in an exogenous assurgent manner to erect branches 1–3–(7) cm. high and 160–250 μ in diam., with main axes distinct but not prominent, and sparingly branched; branches exogenous, arising on all sides; trichoblasts 1–3 times forked, one per segment, arising in a left hand spiral with one fourth divergence, soon deciduous, leaving persistent scar-cells; branches replacing trichoblasts, not arising in connection with them; first trichoblast on a branch arising (1)–3–4 segments from the branch base; tetrasporangia up to 70 μ diam., spirally arranged in the somewhat bulging branch tips; cystocarps ovoideo-globose, sessile, 300–350 μ in diam.; antheridial branches arising from one fork of a trichoblast, measuring 100–170 x 35–40 μ , without sterile tips; plants medium to dark brown, drying nearly black, growing in the middle to upper littoral zone along the coast of southern California and Lower California, and possibly present in the Gulf of California.

Type: Hollenberg 2115, from rocks, Laguna Beach, Orange County, California, May 14, 1937 (H.). Additional representative specimens studied include: La Jolla, San Diego County, California, from boulders at low water mark, Mrs. M. S. Snyder, Phyc. Bor.-Amer. 246, issued as *Lophosiphonia willum* (J. Ag.) S. & G. (C., N. Y., Fi.); and from the

bay side of Punta Banda, Lower California, Mexico, Hollenberg 2563, Dec. 1938 (H.).

P. simplex is very common on sand-swept rocks in the middle littoral zone along the coast of southern California. It seems to be a perennial. In structural details it is close to *P. Snyderae* Kylin, as has been pointed out by Kylin (1941) in his discussion of Phyc. Bor.-Amer. 246. It differs from the latter species in habitat, in the densely matted habit with its much more extensive prostrate system of branches, and in having segments rarely longer than broad. In some cases the trichoblast primordia remain unicellular and directly constitute the scar-cells, as frequently occurs also in other species. In a number of detailed respects *P. simplex* is close to *P. ferulacea* (Suhr.) J. Ag., which has been recorded by Taylor (1928) from Florida and from the Danish West Indies by Boergesen (1919), but differs from the latter plant as described by these investigators in the low matted habit, in its slightly longer segments, and in the lack of sterile tips on the antheridial branches.

The writer has been privileged to examine a number of species of *Polysiphonia* collected by Mr. E. Yale Dawson in the Gulf of California. Nine of these the writer has placed provisionally with *P. simplex*. In Dawson's plants the segments are all very short, mostly less than half as long as broad. Hence they might better be placed with *P. ferulacea*, were it not for the low matted habit of *P. simplex*. Of special interest is the fact that in Dawson's number 284 from Puerto Refugio two tetrasporangia commonly occur in each segment. The other specimens are seemingly sterile.

POLYSIPHONIA *Masonii* Setchell and Gardner, Mar. Alg. Revill. Is., Proc. Calif. Acad. Sci. 19:160, 1930. Plants 2–3 cm. high, flaccid, attached by a bundle of short unicellular rhizoids with discoid tips; main axes 350–400 μ in diam. below, repeatedly and regularly dichotomously branched; pericentral cells 4, ecorticate, composed of segments hardly longer than broad in the main axis, shorter above; trichoblasts dichotomously or trichotomously branched, arising one per segment in spiral arrangement with $\frac{1}{4}$ divergence, deciduous, leaving persistent scar-cells; relation of trichoblasts to origin of branches not observed; tetrasporangia in spiral series in the ultimate branchlets, 85–95 μ in diam.; cystocarps subspherical, nearly sessile, 300–325 μ in diam.; antheridial branches arising from a primary branch of a trichoblast, broadly fusiform; plants reddish brown, epiphytic.

Type: H. L. Mason 86, on *Zostera*, Guadalupe Is., west coast of Mexico, April 1925, in the herbarium of the California Academy of Science. The above description is largely adapted from Setchell and Gardner's description after an examination of the type and only known material. In many detailed characteristics this plant is close to *P. Snyderae* Kylin, but the regularly dichotomous branching throughout and the somewhat discoid attachment organ, together with the lack of additional material, have made it

seem desirable to retain the name as a distinct entity at least for the present.

POLYSIPHONIA *flaccidissima* sp. nov. (fig. 8).—Plantae 10–20 mm. altae a filamentis repentibus; partibus prostratis 70–85 μ crassis, e segmentis fere 1.5 diametros longis, per rhizoidia unicellularia affixae; rhizoidiis variis longitudine, cum apicibus lobatis aut sine lobis, fere unum per segmentum, a finibus proximalibus cellulorum pericentralium abscissis; ramis erectis 70–80 μ crassis, cum segmentis 1–2 diametros longis et sursum brevioribus, exogenibus et assurgentibus a partibus repentibus, cum principibus axibus indistinctis; ramis aliquot vel multorum ordinum, omnibus exogenibus, cum trichoblastis in origine conjunctis ad utrumque terminum gradatim angustis, primum plerumque stristis et ad axem principem curvatis; cellulis pericentralibus 4, omnino ecorticate; trichoblastis simplicibus aut fere cum furcis singularibus saepe ad apicem incurvatis aut spiralibus, fere cum apicibus obtusis, una per segmentum in spira laeva cum $\frac{1}{4}$ declinatione, mox deciduis, cicatricellulas persistentes relinquentes; tetrasporangiis 50–70 μ crassis, in seriebus continuis aut interruptis, plerumque spiraliter in ramulis ultimis et subultimis ortis; cystocarpiis et ramis antheridialibus ignotis; plantae rubrae fere ad saxa et algas corallinas affixae.

Plants 10–20 mm. high from creeping basal filaments; prostrate portions 70–85 μ in diam., the segments mostly 1.5 diam. long, attached by unicellular rhizoids of variable length; rhizoids with or without lobed tips, usually one per segment, commonly two segments apart and cut off from the proximal end of the pericentral cells; erect branches 70–80 μ in diam., their segments 1–2 diam. long and shorter above, exogenous and assurgent from the creeping filaments, with indistinct main axes; branches of several to many orders, all exogenous and arising in connection with trichoblasts, narrowed gradually at both ends, at first usually strict and curving toward the main axis; pericentral cells 4, totally ecorticate; trichoblasts simple or mostly with a single fork; tapering only slightly to the blunt tips, which are often incurved or coiled, arising singly per segment in a left hand spiral with $\frac{1}{4}$ divergence, soon deciduous, leaving persistent scar-cells, or the scar-cells sometimes arising directly from undeveloped trichoblast primordia; tetrasporangia 50–70 μ in diam., in continuous or interrupted and usually spiralling series in the ultimate and subultimate branches, which are more or less swollen, each sporangium appearing above and spirally to the left of the scar-cell immediately below; cystocarps and antheridial branches unknown; color red; plants occurring on rocks and corallines, mostly in exposed places near high tide level along the coast of southern California.

Type: Hollenberg 2269, growing on corallines in shallow depressions on a large wave-swept rocky point at Laguna Beach, Orange County, California, Jan. 1, 1938. It is partly tetrasporic. The species in its typical diminutive form is represented by a number of additional collections by the writer at the same

locality and at other places, including a collection from Catalina Island and a collection as far south as Punta Banda, Lower California, Mexico. The latter collection was made on the bay side of the point in more sheltered water. All collections were made during the months of November to April inclusive and observations throughout the various seasons of the year at the type locality indicate that the plant is an annual at least as far as its appearance in the upper littoral zone is concerned, although var. *Smithii* was collected in the Monterey region in August.

P. flaccidissima is in general similar to *P. havanensis* Montagne as figured by Boergesen (1919, fig. 260, 261). The West Indian plant, however, is described as considerably larger, with leading branches more distinct, and with rhizoids continuous with the pericentral cells bearing them. Furthermore, the relative positions of the young branches and associated trichoblasts are seemingly different in the two species, and the Pacific coast plant rarely if ever bears adventitious branches as described by Boergesen for *P. havanensis*.

P. flaccidissima var. *Smithii* var. nov. (fig. 19).—Plantae 3–5 cm. altae, fere laxius ramosae quam in specie, cum axibus principibus ad 140 μ crassis et cum segmentis (2)–4–6 diametros longis; ramis fructiferis ignotis; plantae sublicis et saxis et conchis fere in aqua placida affixae.

Plants 3–5 cm. high, mostly more laxly branched than in the species, with main axes up to 140 μ in diam., segments (2)–4–6 diameters long; fruiting unknown; plants attached to piling, stones and shells in sheltered or deep water along the coast of central and southern California.

This variety is distinguished by its more luxurious habit, with larger branches and longer segments, and by the habitat. Number 1146 in the herbarium of the writer is considered the type collection. This collection was made from shells and stones near the highway bridge, Newport Bay, Orange County, California, Dec. 8, 1935. Three additional collections have been made: LaJolla, San Diego County, California, from wharf piling at low tide level, Hollenberg 1965, Dec. 1936 (H.); Pacific Grove, Monterey County, California, dredged from rocks at 35–40 ft., G. M. Smith, Aug. 1941, (Hop.); and San Francisco, from floats of Yacht Club, Feb. 1932, N. L. Gardner 6984 (C.).

POLYSIPHONIA Snyderae Kylin, Californische Rhodophyceen, Lunds. Univ. Årsskr. N.F. Avd. 2, 37:35, Taf. 12, fig. 34, 1941; (fig. 9); *Polysiphonia senticulosa* Snyder in Phyc. Bor.-Amer. 638, False Bay, San Diego, California, Jan. 1899, non *Polysiphonia senticulosa* Harvey.

Plants 5–12 cm. high, from a discoid base or mostly assurgent from a prostrate creeping base of limited extent, attached by unicellular rhizoids of variable length and mostly with lobed tips, arising 1–2–(5) per segment from the proximal ends of the pericentral cells from which they are cut off by a cross wall; erect branches 300–400 μ in diam. below, the

segments (0.5)–1–2–(3) diameters long, richly dichotomously branched and mostly naked below, the chief branches repeatedly alternately branched in a more or less dichotomous corymbose manner; pericentral cells 4, without cortications; trichoblasts numerous, arising one per segment in a left hand spiral with $\frac{1}{4}$ divergence, 2–3 times forked, soon deciduous, leaving persistent scar-cells; branches arising at irregular intervals, commonly 6–10 segments apart, in the trichoblast spiral, a branch completely replacing a trichoblast; tetrasporangia 60–70 μ in diam., in segments mostly one diameter long or slightly longer, in series in the ultimate and subultimate branchlets, slightly spiraling; cystocarps abundant, ovoid to nearly spherical on short pedicels of one segment, 300–350 μ in diam., replacing branchlets or trichoblasts in the spiral; antheridial branches comprising a primary fork of a trichoblast; plants dull or pale reddish brown to dull red, epiphytic on other algae or attached to wood, shells, etc., at low tide level, mostly in sheltered bays, Washington to southern California, and in the Gulf of California and south at least as far as the Revillagigedo Islands off the west coast of Mexico.

P. Snyderae is common in sheltered water of bays along the coast of southern California. In many respects it is similar to *P. simplex* and to *P. acuminata* but the segments in the main branches are distinctly shorter in both of these plants than in *P. Snyderae*. The matted habit of *P. simplex* is also distinctive, and as Kylin (1941) has pointed out, *P. Snyderae* is more dichotomous in its branching and has much less distinct main axes than *P. acuminata*. *P. Snyderae* is also similar in many respects to *P. tongatensis* Harvey, which De Toni (1903) places under *P. mollis* Hook. and Harv. The latter plant was described by Harvey (1847) from Tasmania. No specimens of *P. mollis* were available for examination, but judging by Harvey's description it seems possible that our plant may be identical with that species. In any case the writer is of the opinion that *P. tongatensis* (?) reported by Setchell and Gardner (1930) from the Revillagigedo Islands, and certain specimens in the herbarium of the New York Botanical Garden, which are labeled *P. tongatensis* and were collected in Hawaii, the Society Islands, and the Friendly Islands, are very close to if not identical with *P. Snyderae*. Also Robert E. Coker 124 and 126 (N. Y.) from Peru, referred by Howe (1914) to *P. abscissa* Hook. & Harv. are likewise probably referable to *P. Snyderae*. Furthermore, no 200528 in the herbarium of the University of California, which was collected in Japan and identified by K. Yendo as *P. urceolata* has most of the detailed structural features of *P. Snyderae*. *P. Snyderae* differs from *P. novae-angliae* Taylor in the absence of cortical filaments.

P. Snyderae var. *heteromorpha* var. nov. (fig. 15).—Plantae cum structura et ramis fructiferentibus ut in specie, sed cum ramulis numerosis accessoris et cicatrigenibus in partibus inferioribus axorum primariorum orientibus et aliquanto distinctioribus

quam in specie; ramulis fere brevibus, saepe aliquanto determinatis; plantae tenuiores quam species.

Plants with vegetative and reproductive structures mostly as in the species, but with numerous cicatrigenous branchlets arising in the lower parts of the main axes, which are somewhat more distinct than in the species; ultimate branchlets mostly short and often somewhat determinate; plants known only from the coast of Washington and the vicinity of Vancouver Island, British Columbia.

Type (fig. 15), N. L. Gardner 3837a, from pools in the middle littoral zone about three miles south of Cape Flattery, Washington, on the ocean side, May, 1917 (C. 276664). Several additional specimens in the herbarium of the New York Botanical Garden collected by John Macoun in the vicinity of Vancouver Island, may belong here, although they are coarser. The variety differs from the species chiefly in the numerous cicatrigenous branchlets and in the short, more or less determinate, ultimate branchlets.

P. Snyderae var. **intricata** var. nov.—Plantae cum structura et ramis fructiferentibus ut in specie, sed cum partibus inferioribus implicatis et intertextis.

Plants with the general features of the species but with more intricately branched and tangled filaments toward the base rather than with a single point of attachment as is common in the species.

Type: no. 3307 (Fi.) of Francis Drouet and Donald Richards, afloat four miles east of Guaymas, Sonora, Mexico, Dec. 1940. Several additional specimens are from the same general region. This variety differs from the species in the intricate and tangled

basal branches, the plant forming loose masses in which the individual plants are indistinguishable.

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AUXIN AND CALINES IN SEEDLINGS FROM X-RAYED SEEDS¹

G. F. Smith and H. Kersten

It has been previously established by Went (1938a, 1938b) that certain specific factors other than auxin are involved in the growth of plants. These have been placed in a group of plant hormones termed "calines," having the essential character of being transported through living tissues only. Included are: rhizocaline, obtained from the cotyledons in the case of etiolated pea seedlings, which with auxin is responsible for root formation; caulocaline, formed in the roots, which is necessary in conjunction with auxin for stem elongation; and phyllocaline, a requisite for leaf growth which is auxin independent and, in the case of pea seedlings germinated in the dark, is derived solely from the cotyledons. The schemes of activity of these factors as correlated with auxin

in both light- and dark-germinated pea seedlings are diagrammed in figures 1 and 2.

Originally the presence and the differential character of the calines were indicated by tests in which the supply of one or another of the calines was experimentally removed by the excision of various plant parts. For example, a removal of the cotyledons from etiolated pea seedlings exhibited modifications in the elongation of stems in which growth in length ultimately ceased (Went, 1938a).

Additional evidence for the existence of calines as well as for their habit of transport through living tissues only, was assembled by performing experiments in which various amounts of calines were added by grafting experiments with peas using bases of varieties having different contents of some caline (Went, 1938b). In all instances it was found essential that in a graft union incorporating vascular tissue continuity be accomplished between stock and scion before any influence of the base type is exhibited (Hayward and Went, 1939).

The investigation described in this paper supplies further data for the presence of calines, and especially stresses their differential character as they are

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TABLE 1. Maximal growth rate, leaf and stipule surfaces, petiole and tendril lengths, of first, second and third leaf developed in Alaska pea seedlings of control seeds and of seeds given a small x-ray radiation treatment (30 kv, 10 ma, 8 cm. fifteen min.) after two weeks germination. Values given are the mean of forty plants.

	Length	Leaf Width	Surface	Petiole Length	Length	Stipule Width	Surface	Tendril Length	Maximal growth per day mm.
<i>Control:</i>									
First leaf	6.1	3.9	23.8	6.3	4.3	3.1	13.3	2.9	40.2
Second leaf	7.7	4.8	37.0	8.1	6.1	3.9	23.8	5.2	
Third leaf	7.3	5.0	36.5	9.9	5.9	3.9	23.0	9.7	
<i>X-rayed:</i>									
First leaf	4.4	2.8	12.3	4.7	3.5	2.7	9.5	1.5	37.5
Second leaf	6.0	4.0	24.0	6.9	4.8	3.4	16.3	3.8	
Third leaf	6.6	4.7	31.0	10.1	5.5	3.8	20.9	8.3	

involved in the growth of both intact and grafted seedlings. One variety of pea seedling, Alaska, has been used, in which it has been found possible to alter the content of one or another of the calines in the seed by treating the seeds with x-rays prior to germination. Tests to determine auxin conditions in seedlings from x-rayed seeds are also given which indicate results comparable to those obtained by Skoog (1937) in his study on the effect of x-rays on auxin and plant growth.

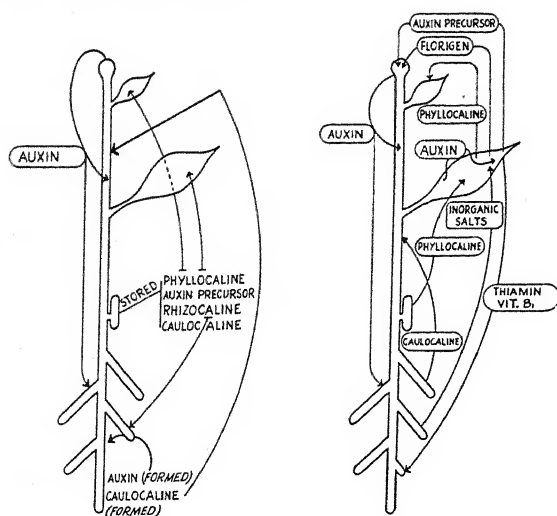


Fig. 1-2.—Fig. 1 (left). Factors involved in dark-room-germinated peas.—Fig. 2 (right). Factors involved in light germinated peas.

METHOD.—For the x-ray treatment of the seeds, soft x-ray radiation was supplied by a copper target gas x-ray tube, equipped with a window of aluminum and cellophane which transmitted the $K\alpha$ and $K\beta$ lines of copper as well as the longer and shorter wavelengths in the continuous spectrum (Kersten, 1934). Because of the great amount of plant material required for the investigation, the seeds were oriented at random during treatment at a distance of 8 cm. from the focal spot of the x-ray tube which

was operated at 30 peak kv. and 10 ma. Under these conditions the intensity of the x-ray beam was approximately 2000 r/min.

The method of random orientation of course does not yield uniformly modified plant material because the side turned toward the target receives many times the dose received by the one turned away from the target. Since the pea embryo is so constructed that the plumule and radicle lie to one side, the effect of random orientation was that certain parts of the individual seeds received treatments ranging from very heavy to relatively very light. The variety of resulting modifications, however, was found to be of positive value during these particular experiments. Definite groups of commonly met x-ray induced plant abnormalities such as retarded stem, root or leaf growth could be uniformly assembled and studied by use of one x-ray treatment. A series of x-ray treatments employing oriented seeds would have been necessary were it desired to associate a specific plant modification with a specific dose of x-ray radiation. This information, however, is generally well known as is also the fact that any x-ray induced effect using random seed orientation can be produced more or less uniformly by some one x-ray treatment of oriented seeds.

All procedures were carried out in a physiological dark room, 24–26°C. and 85% humidity. The pea grafting technique employed was that described by Went (1938b). One-week-old etiolated pea seedlings were used. The stem portion between mid-first and mid-third internodes was removed by two oblique cuts made at the same angle by use of a small mitre box-like structure through which a razor blade could be passed over the stem. The cut surfaces of the remaining stem portions were brought into contact through a 5 mm. piece of glass tubing having a bore which approximated the diameter of the seedling stem.

After the graft preparation, measurements of the stem lengths were taken over a period of eighteen to twenty days, by which time maximal growth had been achieved in most cases; and at this time leaf, stipule, petiole and tendril measurements were generally recorded according to purposes of the par-

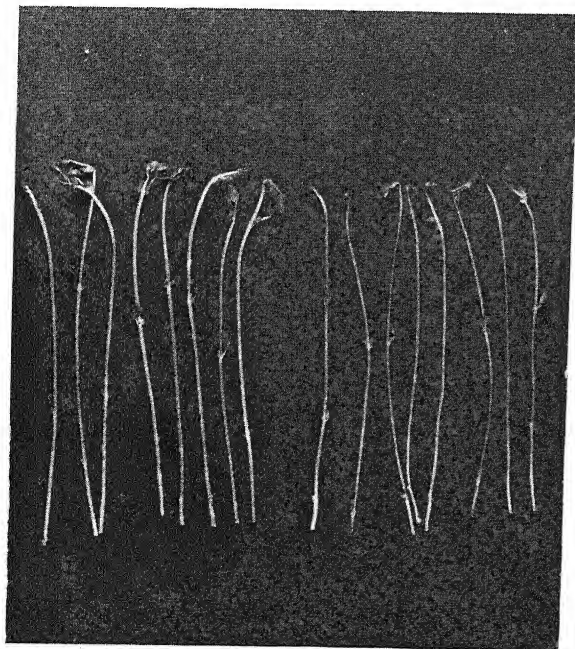
ticular experiment. Leaf measurements were supplemented by results of bio-assays for leaf growth factors as outlined by D. M. Bonner (1940) and by D. M. Bonner, A. J. Haagen-Smit and F. W. Went (1939). Auxin conditions were determined by the standard *Avena* test as specified by Went and Thimann (1937).

Throughout the paper, terms such as x-rayed or treated seedlings, tops, or bases, will be used to refer to seedlings or parts of seedlings from x-rayed seeds.

GROWTH OF INTACT SEEDLINGS WITH X-RAY MODIFIED CALINE CONTENT.—A number of experiments were run with Alaska peas from seeds given brief x-ray exposures. The treated seedlings as well as an equal lot of control seedlings were germinated individually in 20 cc. bottles. Daily growth rates were recorded for eighteen to twenty days, by which time maximal growth had generally occurred. Measurements of leaf, tendril, petiole and stipule of the first, second and third leaf developed were taken. Table 1 lists data from a typical test in which the exposure time was fifteen minutes. Leaf and stipule surfaces were calculated by determining the products of mean lengths and mean widths.

Data presented in the table indicate that the maximal growth rate value for both treated and normal intact seedlings, is relatively constant. Normal variations in Alaska peas generally fall within the range of 34–42 mm. per day, so that the slightly lesser value in the x-rayed set is probably of no experimental significance.

There is, however, a definite decrease in the size of foliar parts of all seedlings from the x-ray treated seeds. This is illustrated in figure 3.



GRAFTING EXPERIMENTS WITH PEAS FROM CONTROL SEEDS AND FROM X-RAYED SEEDS.—Later experiments were carried on with seedlings obtained from seeds exposed to x-rays for sixty minutes while oriented at random. Reasons for using this method have already been given. The types of seedlings obtained from seeds treated in this way can be arranged into four arbitrary groups, figure 4. These are: (1) normal maximal growth rate, reduced leaf size, normal root development; (2) reduced maximal growth rate (about one-third normal), reduced leaf size, normal root development; (3) reduced maximal growth rate (about one-tenth normal), normal root development; (4) reduced maximal growth rate (about one-tenth normal), poorly developed roots.

Grafting experiments with seedlings from group 1, employing various combinations of tops and bases of control and treated seedlings, gave results shown in table 2. The following effects are indicated: (1) uniform maximal growth rates for intact and grafted control and treated seedlings; (2) similar size of foliar parts of control-intact and of control-self-grafted seedlings; (3) similar size of foliar parts of x-rayed-intact and x-rayed self-grafted seedlings; (4) reduced size of all foliar parts of x-rayed-intact and of x-rayed-self-grafted seedlings as compared with values given for control seedlings; (5) decreased size of foliar parts of control tops grafted on x-rayed bases, as compared with normal leaf size; (6) increased size of foliar parts of x-rayed tops grafted on control bases, as compared with normal leaf size.

Apparently the effect of this particular x-ray seed treatment is on the phyllocaline supply which, in

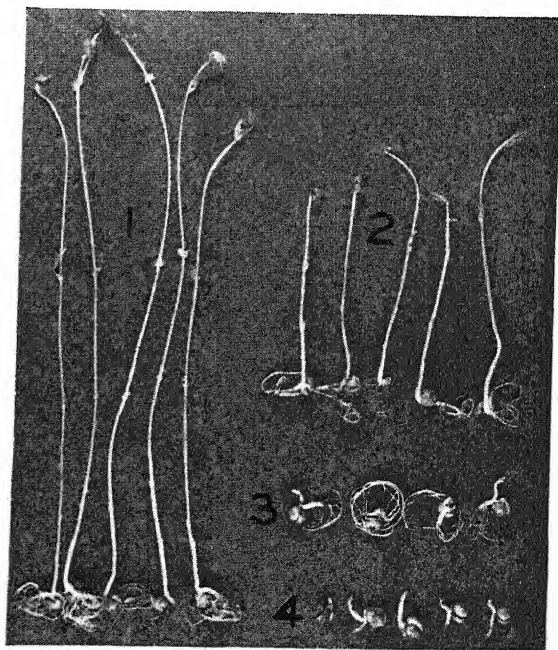


Fig. 3–4.—Fig. 3 (left). Seedling tops from control seeds (left) and from seed given a fifteen-minute exposure to x-rays having an intensity of approximately 2000 r/min.—Fig. 4 (right). Seedling groups from seeds oriented at random while being exposed for sixty minutes to x-rays having an intensity of approximately 2000 r/min.

TABLE 2. Maximal growth rate, leaf surface, stipule surface, petiole and tendril lengths of first, second and third leaves of intact and grafted seedlings of control seeds and of seedlings grown from x-rayed seeds referred to as group 1 in figure 4. Mean values given represent fifteen to thirty plants.

	Leaf			Petiole	Stipule			Tendril	Maximal growth per day mm.
	Length	Width	Surface	Length	Length	Width	Surface	Length	
<i>Control intact:</i>									
First leaf	6.2	3.9	24.2	6.5	4.3	3.2	13.8	3.2	51.5
Second leaf	7.8	4.9	38.2	8.8	6.5	3.9	25.4	5.9	
Third leaf	8.7	5.8	50.5	10.5	6.3	4.2	26.5	9.3	
<i>X-rayed intact:</i>									
First leaf	4.1	3.0	12.3	4.3	3.6	2.7	9.7	1.5	39.8
Second leaf	5.3	3.6	19.1	5.4	4.7	3.5	16.5	3.8	
Third leaf	6.2	4.2	26.0	6.7	5.3	3.7	19.6	7.1	
<i>Control self-grafted:</i>									
First leaf	6.0	3.9	23.4	6.8	4.6	3.4	15.6	3.1	40.5
Second leaf	7.8	4.7	36.7	8.6	5.9	3.8	22.4	5.4	
Third leaf	9.0	5.8	52.2	8.5	6.6	4.6	30.4	10.4	
<i>X-rayed self-grafted:</i>									
First leaf	4.2	2.9	12.2	4.9	4.0	3.1	12.4	1.8	40.8
Second leaf	5.9	3.7	21.8	5.9	4.8	3.5	16.8	3.5	
Third leaf	6.1	4.3	26.2	6.5	5.4	3.8	20.5	6.9	
<i>Control on x-rayed:</i>									
First leaf	6.5	4.0	26.0	5.8	4.9	2.9	14.2	3.7	41.4
Second leaf	6.2	4.0	24.8	6.0	5.3	3.7	19.6	3.9	
Third leaf	6.3	4.3	27.1	6.0	5.5	3.5	19.3	6.7	
<i>X-rayed on control:</i>									
First leaf	5.4	3.5	18.9	5.3	3.3	2.6	8.6	1.7	39.1
Second leaf	7.2	4.4	31.7	6.8	5.3	3.6	19.1	4.5	
Third leaf	7.7	5.1	39.3	9.0	6.5	4.2	27.3	8.8	

dark-room-germinated seedlings, is available solely from the cotyledons. To check this conclusion further, assays for leaf growth factors were run, using methods described by D. M. Bonner, A. J. Haagen-Smit, and F. W. Went (1939). Seed diffusate of Alaska peas was used as a source of leaf growth factors. Circular disks of leaf tissue, 19.5 mm² in area, were cut from young primary leaves of radish. Twelve such disks were floated on 2 cc. of a culture medium in Syracuse watch glass dishes and grown for thirty hours at 25°C. Total wet weight of all twelve sections was determined by direct weighing. The culture medium consisted of 1 per cent sucrose plus various concentrations of a standard solution of pea diffusate, a standard solution having a dry weight of 10 mg. per cc. Diffusates of control and of x-rayed seeds were tested. Results of typical experiments are given in figure 5. Values given represent eight to sixteen assays.

In the assays using diffusate of seeds x-rayed for two hours before extraction, there is little if any leaf activity for concentrations of 0.5, 1.0 and 2.5 mg. dry weight diffusate per cc. of 1 per cent sugar over that obtainable in cultures on the basic 1 per cent sugar solution with no added diffusate. Some increase above the sugar value is meagerly represented when a dilution of 5.0 mg. of this diffusate is employed.

Diffusates of seeds x-rayed for four, six, and eight hours exhibit essentially no activity in promoting

leaf growth above or below the values recorded for the basic sugar medium in the concentrations tested (fig. 5). These extended x-ray treatments were included in the investigation to remove the possibility that the failure of the x-rayed seed extracts to support leaf growth might be ascribed to the production of some toxic substance in the cotyledons which prevented an increase in the size of the cultured leaf disks. If this rather than phyllocaline destruction were responsible, one would expect the effect to be more strongly exhibited with longer x-ray treatments. This does not occur. Also, if the effect of x-raying the seeds were the formation of some substance which inhibited the promotive effect of the seed extract on leaf growth, it ought to be possible to obtain some correlation between the degree of toxicity of such an x-ray produced agent and the concentration of the extract employed, so that the inhibitive effect would become more pronounced with increasing additions of the extract. In every case where higher concentrations are employed, the results fail to substantiate the existence of this relationship. The absence of such an effect is particularly evident in assays using the diffusate of the two-hour x-rayed seeds. As mentioned previously, the cultured leaf tissues responded positively to this extract when employed in dilutions of 5 mg. dry weight per cc. of the basic medium although lower concentrations failed to indicate leaf growth activity.

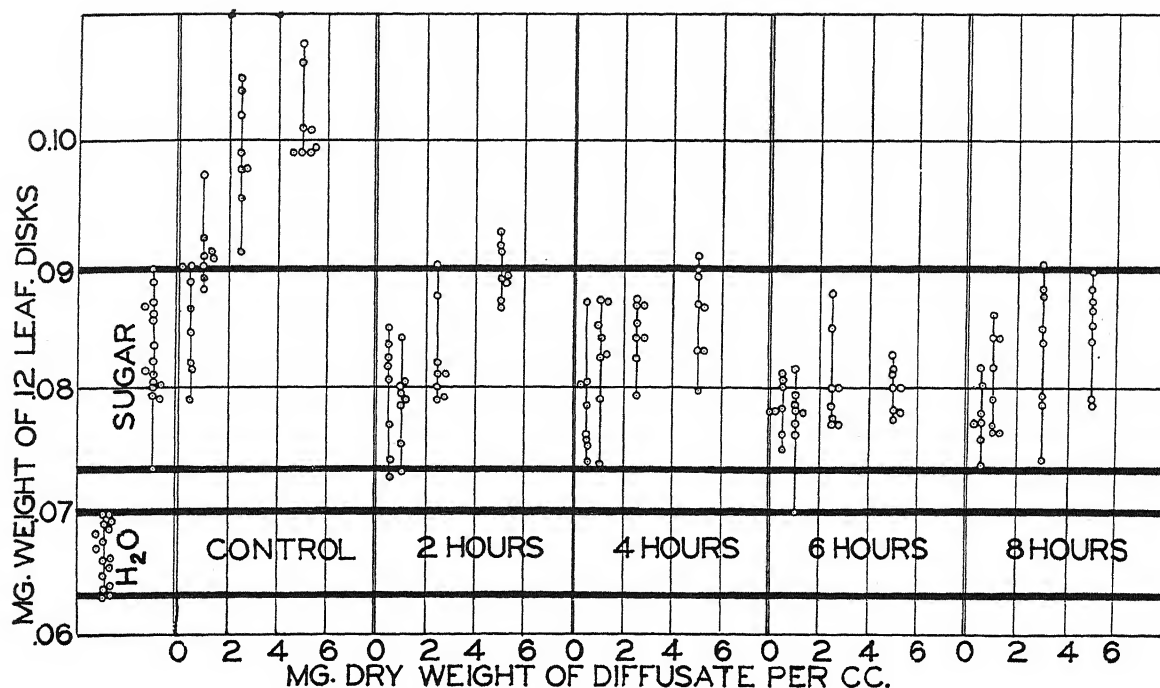


Fig. 5. Results of leaf assays using diffusates from non-irradiated peas and from peas irradiated for two, four, six and eight hours. The horizontal scale indicates the concentration of the seed diffusate employed in terms of mg. dry weight of diffusate per cc. of the basic medium of 1 per cent sugar. The vertical scale indicates the wet weight of the cultured leaf disks. Each value represented by ° indicates the total wet weight of twelve leaf disks. The vertical black lines represent the range of values derived from repeated tests for a particular diffusate dilution. Duplicate values are placed adjacent to each other. The range of growth values found for cultures on water and also for those on 1 per cent sucrose without added diffusate are also shown. In all cases the test period was thirty hours.

TABLE 3. Mean length in mm. of leaf, stipule, petiole and tendril of Alaska pea seedlings from control seeds and x-rayed seeds, the latter seedlings belonging to the group referred to as group 2 in figure 4. Measurements were taken of the first and second leaf developed, after maximal growth had been achieved.

	Leaf	Stipule	Petiole	Tendril	Maximal growth per day mm.
<i>Control intact:</i>					
First leaves	6.4	5.7	6.5	3.6	52.0
Second leaves	9.6	7.3	9.8	5.8	
<i>X-rayed intact:</i>					
First leaves	4.2	4.6	4.0	3.0	14.3
Second leaves	3.9	4.6	3.7	3.5	
<i>Control on control:</i>					
First leaves	4.9	6.2	4.6	4.6	43.0
Second leaves	6.4	6.6	5.0	8.6	
<i>X-rayed on x-rayed:</i>					
First leaves	4.1	4.3	2.8	2.5	45.5
Second leaves	4.1	4.6	3.5	3.1	
<i>Control on x-rayed:</i>					
First leaves	6.6	5.0	5.6	3.3	44.5
Second leaves	6.0	5.3	7.0	3.3	
<i>X-rayed on control:</i>					
First leaves	4.8	4.5	3.6	3.6	43.7
Second leaves	6.7	5.4	8.4	6.6	

Results of grafting experiments with seedlings of the second group of treated plants (fig. 4), characterized by reduced leaf size as well as by reduced maximal growth rate, are listed in table 3. The following results are found: (1) leaf size effects, both in intact seedlings and in various grafting combinations, similar to those observed in the experiments with treated seedlings of group 1; (2) reduced maxi-

used, a reduced maximal growth rate is obtained; (4) when x-rayed base, control interstock, x-rayed top are used, a normal maximal growth rate is obtained.

Experiments designed to test phloem transport through the affected stem portions of treated seedlings by use of externally introduced indicators have been started, but conclusive results are not available to date.

AUXIN CONDITIONS IN SEEDLINGS FROM X-RAYED SEEDS.—Since auxin is also involved in stem elongation in conjunction with caulocaline, its presence was investigated in various stem regions of group 2 treated seedlings (fig. 4). Methods of determining auxin production, transportation, and destruction described previously by Michener (1937) were used. Quantitative results were obtained by the *Avena* test method.

Auxin production, as determined by the auxin diffusion tests from 1 cm. stem sections taken at intervals throughout germination, is found to be less during the first four to six days in the treated seedlings than in controls, but recovery is generally observed by the eighth day (table 5). Auxin transportation tests indicated no effect of the seed treatment.

Auxin destruction was found to be much greater in the stem portions of the treated seedlings than in controls. Typical results are given in table 6. The auxin destroyed is expressed in degrees of curvature of *Avena* coleoptiles (table 6). The effect of auxin destruction in the treated seedlings is seen to increase over controls from the stem apex to the base and to be more pronounced in older seedlings.

The condition of increased auxin destruction in the upper 1 cm. portion seems to have had no impeding effect on stem elongation when employed in grafted combinations of group 2 treated seedlings. The pronounced effect of auxin destruction in the basal portions of the treated seedling stems may, however, have significance in regulating the production of caulocaline or its transport from the roots. No information is available to support this relationship from experiments to date.

TABLE 4. Maximal growth rates in grafted Alaska peas incorporating interstocks using various combinations of control seedlings and of seedlings from x-rayed seeds. Values given are the mean of fifteen to twenty-five plants.

Seedling type	Maximal growth rate in mm./day
Control intact	42.5
Control top, interstock and base	35.9
Control top and base; x-rayed interstock	17.4
X-rayed top, base and interstock	12.0
X-rayed top and base; control interstock	37.7

mal growth rate of intact x-rayed seedlings; (3) normal growth rate of self-grafted x-rayed seedlings.

The only difference between the intact and the self-grafted x-rayed seedlings involved the removal of the mid-first to the mid-third internodal stem portion, as specified by Went's pea grafting technique. Consequently, if the problem were one of stem elongation factors, auxin or caulocaline, it would be a matter of transportation or destruction rather than of content. The significance of this mid-first to mid-third internode stem elongation of the treated seedlings is further indicated in grafting experiments employing interstocks. Representative data are given in table 4. The following results are recorded: (1) when control base, interstock and top are used, a normal maximal growth rate is obtained; (2) when x-rayed base, interstock and top are used, a reduced maximal growth rate is obtained; (3) when control base, x-rayed interstock, control top are

TABLE 5. Effect of x-ray radiation on auxin production in stems of Alaska pea seedlings grown from control seeds and from seeds given a medium x-ray treatment (30 kv, 10 ma, 8 cm, 60 min.) indicated by auxin diffusion tests made at intervals during germination. The treated seedlings were of the type referred to as group 2 in figure 4.

Plant	Age	Tip	Stem part middle	Base	0 γ IAA/L	50 γ IAA/L
Control	4 days	4.0° ^a	3.2°	1.0°	0°	12.3°
X-rayed	4 days	0.3°	-3.6°	1.0°		
Control	6 days	6.16°	2.1°	2.4°	0°	18.8°
X-rayed	6 days	1.1°	0.3°	0.0°		
Control	8 days	4.6°	3.8°	3.1°	0°	18.0°
X-rayed	8 days	4.0°	3.0°	5.3°		
Control	8 days	2.2°	0.7°	0.1°	0°	18.8°
X-rayed	8 days	2.3°	2.3°	0.0°		

^a °curvature indicated by *Avena* test.

TABLE 6. *Auxin destruction in stems of Alaska pea seedlings from control seeds, and from seeds given a medium dose of x-rays (30kv, 10 ma, 8 cm, 60 min.) determined at intervals during germination. The treated seedlings were of the type referred to as group 2 in figure 4.*

Plant	Age	Auxin destruction expressed as °curvature 1 cm. stem portions from—			°curvature pro- duced by	
		Top	Middle	Base	0 γ IAA/L	50 γ IAA/L
Control	6 days	0.6°	1.8°	5.7°	0°	18.8°
X-rayed	6 days	1.6°	8.3°	7.0°		
Control	8 days	0.0°	0.0°	3.8°	0°	18.0°
X-rayed	8 days	4.5°	5.4°	6.0°		
Control	8 days	0.8°	2.5°	1.2°	0°	18.8°
X-rayed	8 days	5.2°	6.4°	4.1°		

SUMMARY

A study has been made of the correlation between x-ray induced modifications and the conditions of auxin and calines in dark-room-germinated pea seedlings from seeds given x-ray treatments. The effects of treatments on auxin production, destruction and transport were determined by the usual test methods, and quantitative results were obtained by the *Avena* test method. Methods used to determine conditions of the calines included direct measurements of parts of intact seedlings and of grafted seedlings incorporating in various combinations, portions of control and treated seedlings. In the case of phyllocline, direct determination of the x-ray effect was made by testing diffusate from x-rayed seeds for leaf activity using the assay method of D. M. Bonner, A. J. Haagen-Smit and F. W. Went (1939).

Data indicated that auxin production and transport were relatively unaffected by the seed treatment, while auxin destruction was noticeably increased in seedlings from the treated seeds, particularly in the basal stem portions. Phyllocline was found by all test methods used to be definitely inactivated by the x-ray treatment, the extent of inactivation depending upon the intensity of the x-ray treatment. Seed diffusates from x-rayed peas produced little to no leaf activity in the leaf test. No apparent effect was found on caulocline production or activity, but the translocation of caulocline seemed to be hindered when higher x-ray doses were employed. This effect is concerned with the mid-first to mid-third internode region of the stem, since recovery in growth rate results in grafted seedlings in which this stem region has been removed. Various graft combinations, employing interstocks of this stem re-

gion taken from seedlings derived from x-rayed seeds, also show retarded stem growth.

The investigations described seem to indicate that the general types of x-ray induced structural modifications described thus far for plants can be explained on the basis of growth-factor destruction or transport inhibition. There seems to be no evidence to date supporting the view that growth inhibitors are synthesized by x-ray irradiation.

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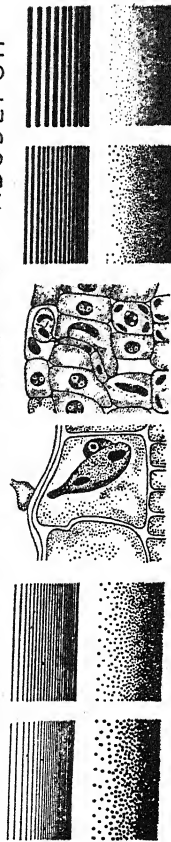


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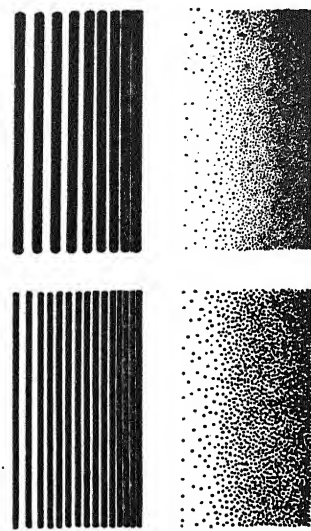
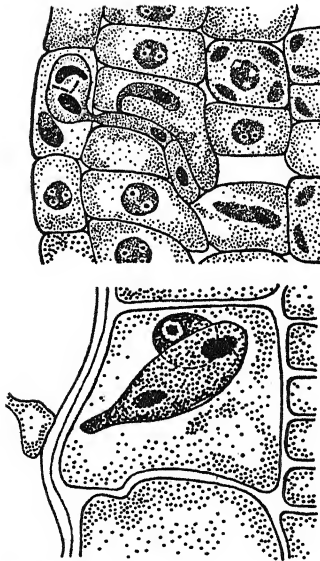
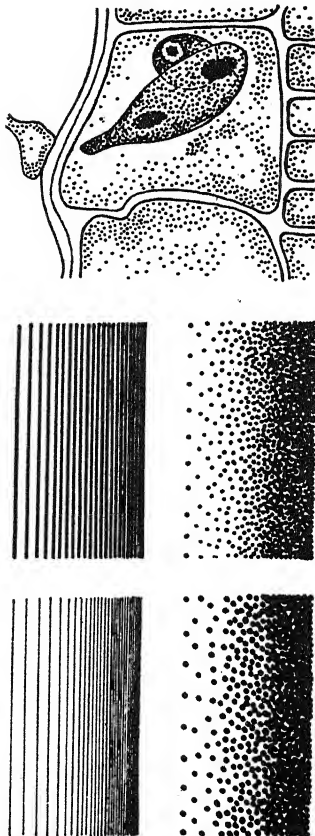


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DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to $\frac{1}{4}$. Middle—Reduction to $\frac{1}{2}$. Bottom—Original size.

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Note that thin black lines hold up fairly well in reduction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. Keep the shading rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

THE EFFECT OF COLAMINE ON GROWTH AND PROTOPLASMIC STREAMING IN AVENA¹

Beatrice Marcy Sweeney

AS FAR as is known, there is no substance in animals comparable to the auxins which control the growth of plants. Extracts of animal tissues, however, contain substances which inhibit the growth of animal cells in tissue culture. Among these inhibiting substances is colamine, or ethanol-2-amine.

The history of the study of inhibitors extracted from liver is surveyed by Medawar (1937). In brief, liver extracts have been known for some time to have an inhibitory effect on cells in tissue culture. In 1926, Heaton made the first exhaustive studies of this factor in aqueous or dilute alcoholic extracts of adult liver. The extracts inhibited the growth of chick heart fibroblasts but had no effect on a number of different epithelial tissues. Heaton also extracted a similar factor from brewer's yeast, but the inhibition was probably due to malt present in the yeast extracts, since Heaton himself later showed that extracts of malt contained active inhibitors of this kind.

Substances inhibiting the growth of mesenchyme but not epithelium have been extracted from germinated and ungerminated barley, from the seeds of maize, wheat, and oats, from whole grain flour and even whole grain bread. The inhibitor has also been obtained in large quantities from whole oranges.

All the extracts contained yellow pigments in varying amounts, but removal of the pigment did not affect the activity of the inhibitor.

Brues and co-workers (1936) have recently studied the effect of these growth inhibiting factors on animal tissue cultures. They used saline or alcoholic extracts of liver to treat different embryonic and sarcomatous tissues. The radius of growth of cultures of fibroblasts was greatly diminished and there was a decrease in the wandering monocytes, although the life span of the treated cultures was not affected and rare mitoses could be found. Cells were smaller in treated than in untreated material. Epithelium was not affected. Brues et al. (1940) later identified colamine in the inhibitory extract. Colamine produced inhibition of growth in concentrations of 0.25 to 2 mg. per cc. A number of other amines were also active. The following study was undertaken to determine whether colamine has any effect on growth in plants comparable to its action on animal tissues, and to study the effect of colamine on protoplasmic streaming in plants, more especially to study its effect in the presence of auxin.

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The author wishes to express her appreciation to Professor K. V. Thimann for his very helpful suggestions and criticisms during the course of this investigation.

METHODS.—Seeds of *Avena sativa* Segerhavre strain were "husked" and soaked for two to four hours in tap water, and then laid out to germinate on moist filter paper in petri dishes. The plants were then grown in a dark room at 24°C. and 85 per cent relative humidity with occasional red light. Unless otherwise stated, the seedlings were used when four days old from soaking. The coleoptiles were then slit lengthwise and arranged on a slide and covered with a coverslip, so that the cells of the outer epidermis could be observed. The solutions used were added at one side of the coverslip and removed from the other side by a filter paper wick, so that a flow of solution under the coverslip was maintained.

The auxin used was recrystallized synthetic indole-3-acetic acid. The colamine was a synthetic preparation obtained from Hofmann La Roche. Dilutions of auxin and colamine were made up in 1 per cent fructose (Sweeney and Thimann, 1938). Unless otherwise stated, the solutions were oxygenated, and the pH was adjusted to neutrality (6.8) with HCl.

Streaming measurements were made by automatic recording (Sweeney and Thimann, in press) as well as with stop watch and ocular micrometer. Measurements of the effect of auxin and colamine together were repeated from time to time in three successive years.

Growth measurements were made using the section test of Schneider (1938). Sections of a known length were cut from the coleoptile and placed on combs floated on the solution to be tested. After varying periods of time, the length of the sections was measured with a microscope fitted with an ocular micrometer.

EXPERIMENTS ON PROTOPLASMIC STREAMING.—*The effect of colamine itself.*—Colamine in concentrations below 1000 mg. per liter did not bring about any consistent change in the rate of streaming. At a concentration of 10,000 mg. per liter, colamine brought about a fall in the rate of streaming and usually stopped streaming completely in fifteen to twenty minutes. Since colamine is a strong base, careful control of the pH was necessary. When the pH of the solution was not adjusted (pH 9) and no oxygen was bubbled through the solution, colamine concentrations of 1000 mg. liter also caused a decrease in the rate of streaming (fig. 1, curve 2), but either oxygenation or adjustment to pH 6.8 prevented this decrease. The effect of high concentrations of colamine was not due to high pH alone, however, since in solutions of NaOH at pH 9 the rate of streaming was not decreased (fig. 1, curve 4). Potassium malate 0.001M when added to a solution of colamine (1000 mg. per liter at pH 9 in unoxygenated water) prevented the decrease in the rate of streaming (fig. 1,

curve 3). From these experiments, then, no simple explanation may be offered for the effect of high concentrations of colamine. It may be noted that these are the concentrations (0.5 gr. per liter or above) which inhibit the growth of fibroblasts in tissue culture. Brues et al. (1940) also found alkaline solutions to be inhibitory in lower concentrations than solutions near neutrality. In general, the

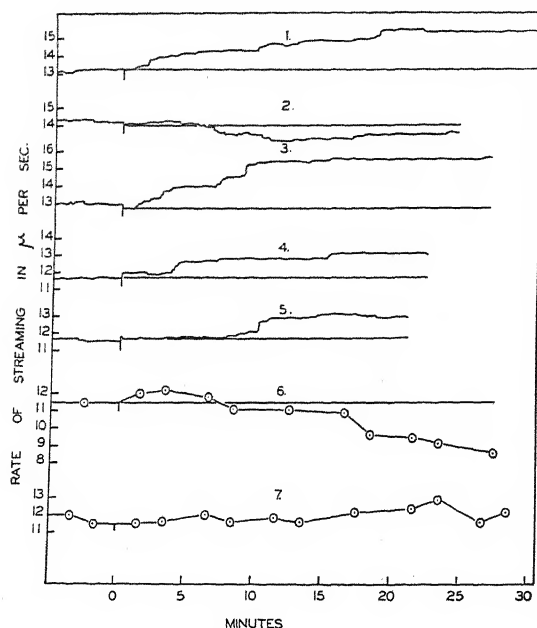
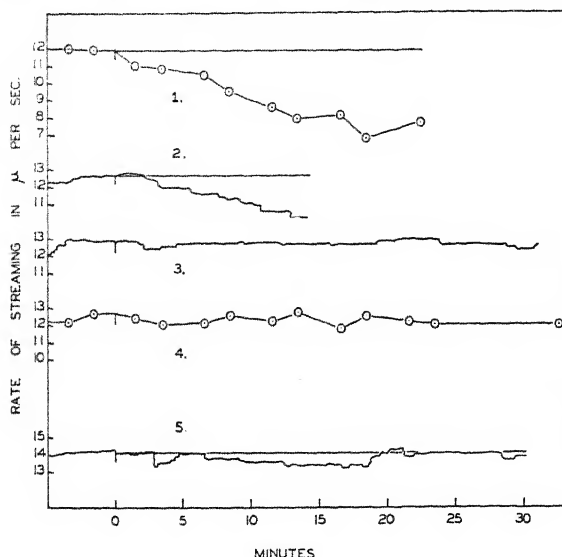


Fig. 1-2.—Fig. 1 (above). The effect of colamine in 1 per cent fructose solution on the rate of streaming in four-day old coleoptiles. Curve 1: colamine 10,000 mg./l in unoxxygenated solution at pH 6.8, (5/4/39). Curve 2: colamine 1000 mg./l in unoxxygenated solution at pH 9, (2/23/40). Curve 3: colamine 1000 mg./l and malate 0.001M. in unoxxygenated solution at pH 9, (2/23/40). Curve 4: sodium hydroxide in unoxxygenated solution at pH 9. Curve 5:

effect of pH could no doubt be ascribed to its influence on the ionization of the colamine, the undissociated substance penetrating the cell more easily than the charged ion $\text{HO}(\text{CH}_2)_2\text{NH}_3^+$.

The effect of colamine with auxin.—In contrast to the high colamine concentrations necessary to produce an effect on the rate of protoplasmic streaming when auxin is not added, colamine in the presence of auxin acts in low concentrations comparable to active concentrations of other catalytic substances. The addition of colamine (3 mg. per liter or above) to solutions containing auxin prevented the stimulation of streaming which is produced in four-day old coleoptiles by auxin in concentrations of 0.01 to 1 mg. per liter (fig. 2, curves 1 and 2). Experiments testing the effect of colamine and auxin together on four-day old coleoptiles were repeated many times, and colamine always prevented the auxin from affecting streaming.

Since the acceleration of streaming by auxin requires ample oxygen supply, it might be suggested that colamine acts indirectly through increasing the oxygen consumption of the cell and hastening the onset of oxygen deficiency for streaming. That this is not the case was shown by experiments in which unoxxygenated solutions of colamine and auxin (1 mg. per liter) were used (fig. 2, curves 6 and 7). Under these conditions, but without the presence of colamine, the rate of streaming is decreased due to lack of oxygen (Sweeney and Thimann, 1938). With colamine present, auxin had no effect on the rate of

colamine 10 mg./l in oxxygenated solution at pH 6.8, (10/31/41). Each curve reproduced here is typical of several such experiments. Curves shown as continuous lines were obtained with automatic recording, those drawn through points were obtained by the stop watch method.—Fig. 2 (left). The effect of colamine in the presence of auxin on the rate of streaming in coleoptiles three to four days old. All solutions were made up in 1 per cent fructose at pH 6.8, and were added to the coleoptile preparation at time zero. Each curve reproduced below is typical of a number of such experiments. Curve 1: four-day old coleoptile treated with auxin 1 mg./l in oxxygenated solution, (7/21/41). Curve 2: four-day old coleoptile treated with auxin 1 mg./l and colamine 10 mg./l in oxxygenated solution, (7/21/41). A comparison of this curve with curve 1 shows that colamine inhibits the effect of auxin on streaming. Curve 3: four-day old coleoptile treated with auxin 1 mg./l, colamine 10 mg./l and malate 0.001M. in oxxygenated solution, (7/21/41). Malate thus prevents inhibition by colamine. Curve 4: three-day old coleoptile, used ninety min. after decapitation, treated with auxin 1 mg./l in oxxygenated solution, (4/4/40). Curve 5: three-day old coleoptiles used ninety min. after decapitation, treated with auxin 1 mg./l and colamine 100 mg./l in oxxygenated solution, (4/4/40). A comparison of curves 4 and 5 shows that colamine does not inhibit the effect of auxin on streaming in three-day old coleoptiles. Curve 6: four-day old coleoptile treated with auxin 1 mg./l in unoxxygenated solution, (1/24/39). Curve 7: four-day old coleoptile treated with auxin 1 mg./l and colamine 10 mg./l in unoxxygenated solution, (1/24/39). Curves shown as continuous lines were obtained with automatic recording, those drawn through points were obtained by the stop watch method.

streaming. Thus colamine simply removes the effect of auxin, whether the latter is positive or negative.

The nature of the effect of colamine on the response of the cell to auxin was made clearer by experiments in which the test solution contained auxin, colamine, and malate. Evidence that the four-carbon dicarboxylic acids, of which malate is representative, play a part in the fundamental reaction brought about by auxin is discussed in a previous paper (Sweeney and Thimann, in press). The effect of malate in four-day seedlings was to remove the inhibition of auxin caused by colamine. Solutions containing auxin, colamine and malate accelerated streaming to the same degree as did controls containing auxin alone (fig. 2, curve 3).

In this connection it is of interest to note that colamine did not inhibit the effect of auxin on the rate of streaming in three-day old coleoptiles (fig. 2, curves 4 and 5). The data of Commoner and Thimann (1941) and the experiments on old coleoptiles reported in a previous paper (Sweeney and Thimann, in press) provide evidence that the supply of four-carbon acids is depleted as the seedling grows. The four-carbon acids present in three-day old plants may be sufficient to overcome the effect of colamine.

GROWTH EXPERIMENTS.—The streaming data suggested that colamine might act as an inhibitor of growth. To test this possibility, growth of coleoptile sections was measured in fructose solutions containing either auxin or auxin plus colamine and in fructose solutions alone. The pH of all solutions was 6.8. Sections were cut from three-day old plants. No inhibition of growth was found in colamine (100 mg. per liter or 10 mg. per liter). Concentrations of 1000 mg. per liter inhibited growth about 50 per cent (see table 1):

TABLE 1. Inhibition of growth by colamine. The auxin concentration used was 1 mg. per liter; colamine, 1000 mg. per liter. All solutions were made up in 1 per cent fructose at pH 6.8.

Per cent elongation of sections after twenty-four hours in:			
Auxin	Auxin and colamine	Sugar alone	Per cent inhibition by colamine
76	33	17	57
60	39	18	35

In several, although not all, of the experiments with low concentrations of colamine, sections treated with auxin and colamine continued to grow for a longer time than those in auxin and, thus, actually reached a greater length (fig. 3). This suggests that the effect of colamine is to spare some substance (not auxin) which is necessary for growth. To test this possibility, preliminary experiments were made in which sections were kept in colamine for a time before being placed in auxin. These sections grew very little, due to aging before the addition of auxin. Heyn (1934) has shown that aging may be pre-

vented by cold. Therefore, sections were pretreated in the dark at 3°C. The sections were pretreated with colamine, auxin, malate and sugar alone for twenty-four and forty-eight hours. At the end of this time, the sections were transferred to auxin or auxin plus malate and allowed to grow at 25°C. Cold

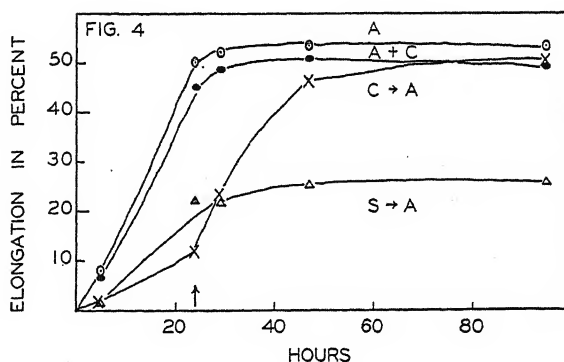
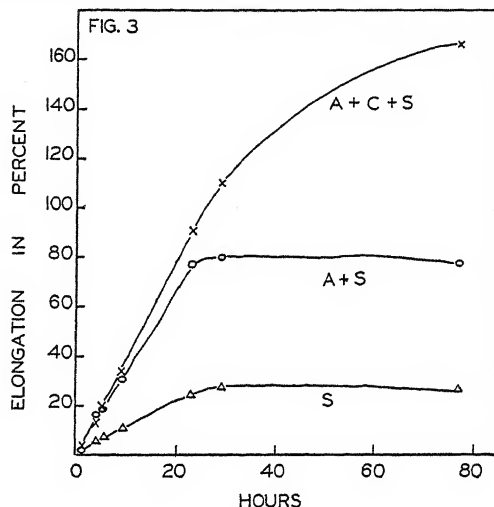


Fig. 3-4.—Fig. 3. The effect of colamine on elongation of coleoptile sections in solutions containing auxin. Three mm. sections cut from etiolated coleoptiles sixty-nine hours from soaking were placed in 50 cc. 1 per cent fructose solution containing auxin 1 mg./l (○), auxin 1 mg./l and colamine 100 mg./l with fructose (×), or sugar alone (Δ) all at pH 6.8. The solutions were renewed after nine hours. Each point is the mean elongation of thirty sections from ten plants, (3/29/40).—Fig. 4. The effect of pretreatment with colamine on the growth of sections subsequently placed in auxin solution. Three mm. sections cut from coleoptiles 71½ hours old from soaking (12/9/40) were placed in 50 cc. of 1 per cent fructose solution containing auxin 1 mg./l (○), auxin 1 mg./l and colamine 100 mg./l (●), colamine 100 mg./l (×), or sugar alone (Δ), all at pH 6.8. After twenty-four hours (at arrow on the abscissa), the sections previously in colamine (see curve marked C→A) or sugar alone (see curve marked S→A) were transferred to auxin solution 1 mg./l and the auxin and auxin plus colamine solutions were renewed. All solutions contained 1 per cent fructose. Each point is the mean elongation of thirty sections from ten plants.

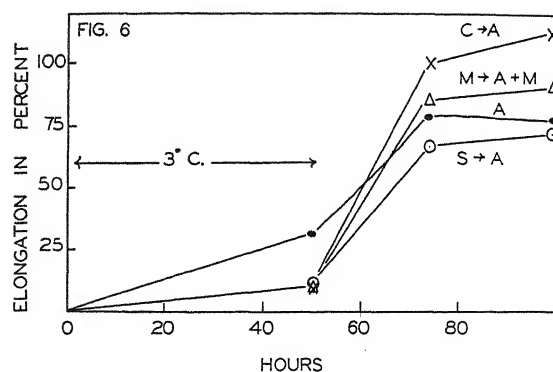
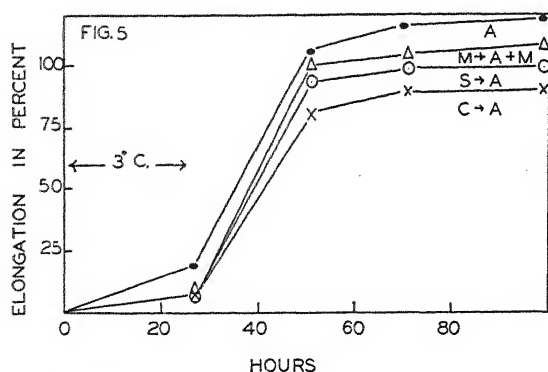


Fig. 5-6.—Fig. 5. The effect of a twenty-six hour cold treatment on elongation. Three mm. sections cut from etiolated coleoptiles 68½ hours old from soaking (9/2/41) and still containing the leaf were placed in 10 cc. of 1 per cent fructose solution containing auxin 1 mg./l (●), colamine 100 mg./l (×), malate 0.001M. (Δ), and sugar alone (○). After twenty-six hours at 3°C. in the dark, sections in colamine or sugar alone were transferred to auxin 1 mg./l, those in malate, to auxin 1 mg./l and malate 0.001M., while sections in auxin were placed in fresh auxin solution. All solutions contained 1 per cent fructose. Further growth took place at 24°C. in the dark. Each point is the mean elongation of thirty sections from ten plants.—Fig. 6. The effect of a fifty-hour cold treatment on elongation of sections. Three mm. sections cut from etiolated coleoptiles 71½ hours old from soaking (9/9/41) and still containing the leaf were placed in 10 cc. of 1 per cent fructose solution containing auxin 1 mg./l (●), colamine 100 mg./l (×), malate, 0.001M. (Δ), and sugar alone (○). After fifty hours at 3°C., sections in colamine and sugar alone were transferred to auxin 1 mg./l, those in malate, to auxin 1 mg./l and malate 0.001M., while sections in auxin were placed in fresh auxin solution. All solutions contained 1 per cent fructose. Further growth took place at 24°C. in the dark. Each point is the mean elongation of thirty sections from ten plants.

treatment considerably slowed the aging process. After twenty-four hours' cold, all the sections including the sugar controls grew as well as three-day old plants upon being removed from the ice box and transferred to auxin (fig. 5). It may be noted in passing that during the cold period, sections in auxin grew significantly more than did those without auxin, showing that growth is not prevented by temperatures which almost completely stop deposition of secondary wall, thus corroborating earlier observations of Heyn.

If the cold treatment was for forty-eight hours (fig. 6), then sections soaked in colamine or malate retained the ability to react to auxin better than did sections soaked in sugar. Aging due to exhaustion of factors necessary for growth seems to be operative here. These results add support to the hypothesis that colamine spares a growth promoting substance, possibly a four-carbon acid.

The question arose as to whether this sparing effect could also be demonstrated by streaming measurements. It was found in other experiments (Sweeney and Thimann, in press) that three-day old coleoptiles, sectioned and soaked in sugar solution or water for twenty-four hours, lost the ability to react to auxin with an acceleration of the rate of streaming. Since streaming is unaffected by thickening of the cell wall, it was concluded that substances other than auxin were depleted during soaking and that, since malate restored the activity of auxin, these lost substances were probably four-carbon acids. Therefore, sections were soaked overnight in colamine (100 mg. per liter), controls being soaked in sugar solution alone. The following day these sections were treated with auxin 1 mg. per liter while

records of the rate of streaming were made (fig. 7). As expected, those coleoptiles, soaked only in sugar solution, showed no acceleration of streaming on the addition of auxin, while sections soaked in colamine responded to auxin with as large an acceleration of streaming as did intact four-day old plants.

DISCUSSION.—In *Avena* coleoptiles, an effect of colamine may be detected at concentrations considerably lower than those inhibiting growth of fibroblasts in tissue culture. The immediate effect of low concentrations of colamine appears from data on streaming to be an inhibition of the effect of auxin, while growth measurements suggest that, after longer periods of time, colamine may act to promote

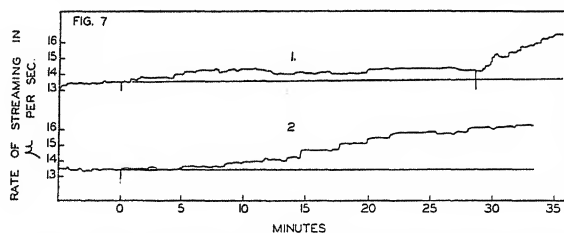


Fig. 7. The effect on the acceleration of streaming caused by auxin of soaking coleoptile sections overnight in colamine. All solutions were made up in oxygenated 1 per cent fructose. Similar results were obtained in two other such experiments. Curve 1: sections from three-day old coleoptiles soaked twenty-four hours in 1 per cent fructose, and treated at time zero with auxin 1 mg./l and at twenty-eight min. with auxin 1 mg./l and malate, 0.001M., (7/19/41). Curve 2: sections cut from three-day old coleoptiles and soaked twenty-four hours in colamine 10 mg./l in 1 per cent fructose solution and treated at time zero with auxin 1 mg./l (7/19/41).

the growth reaction. This contradictory evidence at once suggests that colamine is not simply an auxin inhibitor, but acts indirectly on the growth mechanism, probably through an effect on the availability of the four-carbon acids. A possible mechanism might be the formation of a relatively unstable compound of colamine with one of the four-carbon acids, which may dissociate in time, setting free again the four-carbon acid. This compound is not a salt, since at pH 6.8 a salt would be dissociated immediately. A linkage of the NH_2 of colamine with $\text{C}=\text{O}$ as in oxaloacetic acid is a possibility. The immediate effect of colamine would thus be to remove four-carbon acid from the reaction causing immediate auxin inhibition, and to preserve this acid in the form of a compound from which it is later set free. If this liberation takes place at a time when growth is limited by the four-carbon acids, then colamine will appear to accelerate growth.

The possibility exists that a similar tying up of the four-carbon acids may account for the inhibition of growth of animal cells, in which it is thought (Szent-Györgi, 1937, 1939) that most, if not all of respiration involves the four-carbon acid cycle. Both Medawar and Brues noted extremely rapid growth of inhibited cultures as soon as the inhibiting solution was removed. As in plants, susceptibility to colamine increases with increasing age, both of the tissue and of the culture. It may be suggested that the influence of the addition of malate on the growth of fibroblasts is well worth study.

SUMMARY

Colamine, one of a number of amines which inhibit the growth of fibroblasts in tissue culture, was found

to retard the rate of streaming in oat four-day old coleoptiles, but only at very high concentrations, 1000–10,000 mg. per liter.

Lower concentrations of colamine (3 mg. per liter or more) at pH 6.8 inhibited the acceleration of streaming brought about by auxin in four-day old coleoptiles, both in oxygenated and in unoxygenated solutions.

This inhibition was removed by the presence of 0.001M malate.

Growth studies showed that colamine does not inhibit the growth of sections of three-day old coleoptiles. In solutions of auxin and colamine, growth sometimes continued longer than in auxin alone.

When coleoptile sections were soaked overnight in various solutions, aging being prevented by carrying out this preliminary treatment at 3°C ., their subsequent ability to grow in auxin solution was maintained. If the cold treatment was forty-eight hours, the subsequent growth in auxin of sections pretreated with either colamine or malate was greater than that of sections pretreated with either auxin or sugar alone.

Streaming of coleoptiles cut when three days old and soaked overnight in colamine (100 mg. per liter) was accelerated on the addition of auxin, while coleoptiles soaked in sugar alone did not show acceleration of streaming on the addition of auxin.

It is concluded that colamine acts by making temporarily unavailable a substance necessary for the action of auxin, possibly a four-carbon acid.

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THE USE OF RADIOACTIVE PHOSPHORUS IN TRANSLOCATION STUDIES¹

Robert N. Colwell

ONE of the most difficult problems in the study of rapid solute transport in plants is to limit the solute being studied to a single conducting tissue so that one can accurately observe the factors determining its rate and direction of movement. The work of Mason and Maskell (1928) and of Stout and Hoagland (1939) indicates that a ready transfer of solutes may take place between xylem and phloem by cell-to-cell diffusion accelerated by protoplasmic streaming. By the same means solutes may move longitudinally at rates as high as 2 cm. per hour (Crafts, 1939) through channels entirely distinct from the sieve tubes and vessels that are regarded as the normal conduits for rapid longitudinal movement. Failure to distinguish this accelerated cell-to-cell diffusion from rapid longitudinal transport has led to many questionable interpretations from experiments designed to elucidate the mechanics of translocation. For this reason Caldwell's translocation studies (1936) using viruses have been criticized; also Schumacher's fluorescein experiments (1933, 1937), the work of Fischer (1936), and Both (1937) with nitrogen compounds, and Gustafson and Darken's translocation studies (1937) using radioactive isotopes.

All these experiments have been criticized (Crafts, 1939) because rates greater than those of cell-to-cell diffusion accelerated by protoplasmic streaming were not demonstrated, so that conclusions relevant to the mechanism of rapid solute transport were not justified.

Several workers have used radioactive isotopes as indicators in experiments on solute transport in plants. Gustafson and Darken (1937) have already been mentioned. Stout and Hoagland (1939) used oiled paper to separate bark from wood for a short distance along stems of willow and geranium. They found that radioactive isotopes of potassium, sodium, or phosphorus introduced in the solution around the roots of these plants subsequently appeared in the wood but not in the bark of the separated region. Wherever the phloem was left attached to the xylem, however, it contained the test element almost as soon as the xylem itself. These observations point to the xylem as the sole channel for rapid upward transport of water and salts in the stem below the principal transpiring region. They also indicate the rapidity with which salts diffuse radially from xylem to phloem.

More recently Biddulph (1941) has studied the migration of injected radiophosphorus from bean leaves. He injected the terminal leaflet of the second alternate leaf of each plant as follows. While the blade was held under water he dissected out the

principal lateral vein of the leaflet to form a flap. This flap was immediately dipped into a small container of radiophosphorus, left for five minutes, and then removed to a special container of water. After a few hours he ashed various fractions of the plant, analyzed them with a Geiger counter, and found radioactivity in the stem both above and below the node of the treated leaf. Since the plant was actively transpiring, he concluded that the radiophosphorus had been transported upward in the xylem and downward in the phloem.

The work of Strasburger and Dixon on dye movement in the xylem, however, and more recent work on xylem injection indicate that with Biddulph's injection method no clear differentiation can be made between xylem and phloem movement. His experiments are, therefore, of doubtful significance with respect to the direction, localization, or mechanism of normal solute movement in plants.

In using radioactive isotopes in physiological experimentation one should remember that they may affect cell permeability. As early as 1926 Fricke (1926) showed that beta and gamma rays can denature proteins. Barnett (1939) points out that electrically asymmetric lipoidal molecules such as those found in cell membranes may change their polar properties when bombarded with electrons or gamma rays, and that, according to Wilbrandt (1935), any such change will alter the permeability of the membrane.

Crane (1939) asserts, "It is unnecessary to consider the *local* effect of a particular radioactive atom upon a cell which happens to be close to it." He further states that the effect on permeability depends upon the magnitude of the generalized dose of ionization delivered to the material throughout its volume. The beta rays emitted by radiophosphorus must affect permeability much as x-rays do, since the latter produce rapidly moving electrons. Heilbrunn and Mazia (1936) have reviewed experiments dealing with the effect of x-rays on permeability. In general no effect is noted below an activity equivalent to about 10^6 disintegrations per cc. per second, which is roughly equivalent to a strength of 20 microcuries of radiophosphorus per cc. of solution.

In the experiments to be described, solutions having strengths as high as 100 microcuries of radiophosphorus per cc. were placed on the leaf. By its effect on cell permeability this radioactivity may have facilitated the penetration of radiophosphorus into the leaf, but so little of this substance eventually migrated out of the leaf in these experiments on phloem transport that there was probably little effect on permeability in the conducting elements themselves, if Crane's values are correct. Crane has apparently neglected, however, to consider the probability that plant cells will accumulate radioactive salts from the bathing solution and by thus concen-

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The writer is indebted to A. S. Crafts, D. R. Hoagland, and J. P. Bennett for their advice, and to John Lawrence for generously providing the radioactive phosphorus used in these studies.

trating them will induce a local radioactivity far in excess of this generalized dose of ionization.

Mullins (1939) has studied the rate of uptake of radioactive sodium by *Nitella*. According to him there is no effect on permeability at doses below 1 microcurie per cc. of solution, but at strengths of 1 to 50 microcuries per cc. the rate of penetration of sodium is *inversely* proportional to the log of the dose. He obtained similar results with radioactive potassium and again with x-rays as the radiant source. However, his experiments were run for periods of only a few hours.

As Mullins (1939) has pointed out, the radiations from various radioactive isotopes differ so markedly that a different limit of biological effect must be made for each one. Hevesy (1940) finds little indication of injury to tissues from radioactive indicators when used in low concentrations. In his experiments on red blood cells he calculates that only one Na^{24} atom was used per 1000 cells and that only one in ten of those disintegrated during the experiment. In interpreting results of translocation studies one should certainly consider the radioactivity of the solution used and its consequent effects on cell permeability. At present, however, our knowledge of threshold concentrations and of precise biological effects is inadequate for a detailed interpretation with respect to mechanisms of the type involved in the transport of solutes in plants. Permeability effects are probably more pertinent in absorption studies than in translocation work; in the latter, rates of movement are such that little injury to a single conducting element could occur while the necessary amount of tracer is moving through it. Although often in the experiments described in this paper far more than the necessary amounts of radioactive phosphorus were injected into leaves, no injury attributable to the tracer was found at any time.

EXPERIMENTS ON THE CHANNEL OF TRANSPORT OF RADIOACTIVE PHOSPHORUS.—Hubbard squash plants were grown in the greenhouse in Hoagland's water culture solution with forced aeration. When these were two months old and were growing very rapidly, four healthy ones were selected for experimentation.

Figure 1 shows some of the plants. In plants 2 and 4 (table 1) the petiole of the leaf to be treated was



Fig. 1. Hubbard squash plants used in the experiments on transport of radioactive phosphorus.

first scalded for a distance of about 10 cm. by dipping it in a beaker full of boiling water for approximately one minute. The scalding killed all living cells in the scalded region and by coagulating proteins in the sieve tubes rendered the phloem func-

TABLE 1. *Experiment on the channel of transport of radioactive phosphorus.*

	Plant number			
	1	2	3	4
Treatment of petiole.....	Not scalded	Scalded	Not scalded	Scalded
Time between treatment and collection, hours	40	40	3	3
Approximate area of leaf covered with solution, cm ²	6	6	25	25
Final distribution of radioactive phosphorus	Throughout stem	Confined to treated leaf	Throughout stem	Throughout stem
Apparent channel of transport of radioactive phosphorus in petiole of treated leaf.....	Phloem only	Phloem only	Both xylem and phloem	Both xylem and phloem ^a

^a Except in the scalded region where only the xylem acted as a channel.

tionless for rapid conduction. Apparently, it did not impair conduction in the xylem.

In treating each plant, a plasticine ring was carefully molded on the middle of the upper surface of one leaf; and at 7:30 P.M. into the well thus formed was poured about 10 ml. of an aqueous solution containing a total of 150 mg. of Na_2HPO_4 in which was approximately 1 millicurie of radioactive phosphorus (P^{32}).

The plants were collected after the periods indicated in table 1, and each stem was immediately cut up into segments. To determine the distribution of radioactive phosphorus, cross sections of the stem were cut at various levels above and below the node of the treated leaf, and radioautographs of them were made by placing the sections on a thin piece of aluminum foil directly over a piece of Eastman no-screen x-ray film. If the sections contained no radioactive phosphorus, the film was not affected, but the portion of it immediately beneath any tissue containing radioactive phosphorus was activated.

To secure clarity of the radioautographs, the following steps were taken:

1. The sections were pressed closely to the film with rubber-band wrappings, after first backing the film with cardboard to give it rigidity and placing a piece of paper toweling immediately over the freshly cut sections to absorb moisture squeezed from them.

2. Since x-ray film is very sensitive to light, the sections were mounted in the dark-room and placed in light-tight boxes.

3. For clear radioautographs, exposure periods of forty-eight hours or more were usually necessary. To reduce diffusion of phosphorus within the tissues of the sections during these prolonged exposure periods, cold temperatures were maintained by keeping the light-tight boxes and enclosed films in a refrigerator.

Table 1 gives details of the treatment for each plant.

Since it was desired to compare the final distribution of phosphorus in plant 1 with that in plant 2 by means of radioautographs, the stem sections cut from plant 1 and from plant 2 were placed over a single film in order to give all sections identical exposure and development. For the same reason all cross-sections were cut to approximately equal thickness, 200 microns, in a hand microtome. Each horizontal row contained five serial sections taken from the same level.

Figure 2 shows the results of this experiment as shown by radioautographs. Sections from plants 3 and 4 were radioautographed in a similar manner for comparison.

As figure 2 shows, scalding the petiole prevented export of radioactive phosphorus from the leaf in

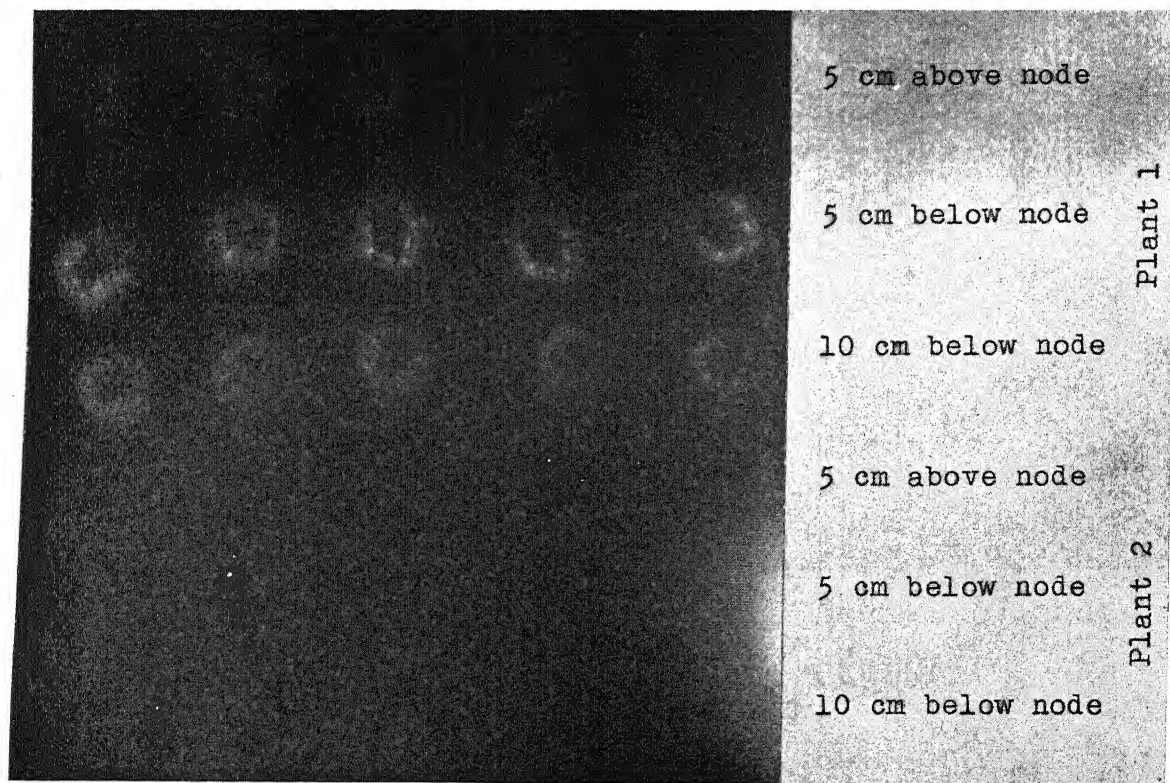


Fig. 2. Radioautographs of stem cross sections from plants 1 and 2 cut at the levels indicated on the right. Sections are 200 microns thick. Exposure period 2 days. Sections from plant 2 though barely discernible on the negative are lost in the process of reproduction.

plant 2 for the entire forty-hour period even though the xylem was still functional. This fact indicates that export of radioactive phosphorus in plant 1 must have been through the phloem.

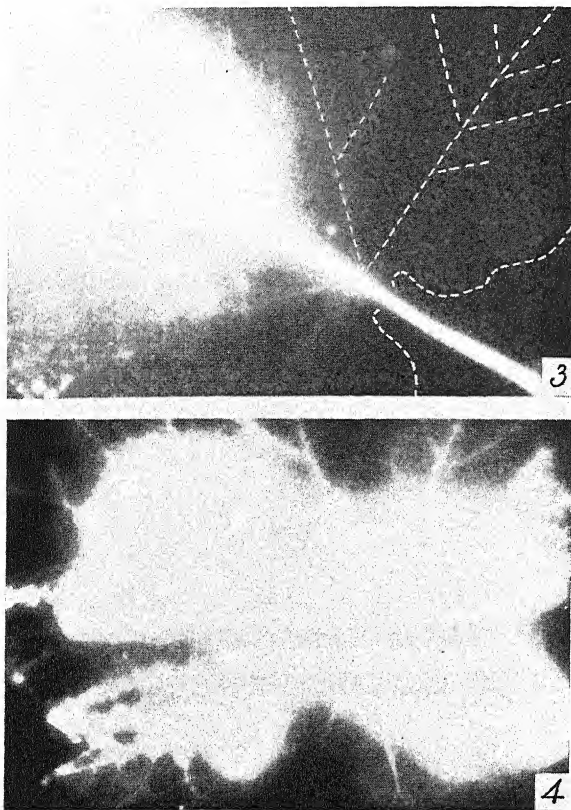


Fig. 3-4.—Fig. 3. Radioautograph of treated leaf from plant 1 with normal petiole. Dashed white lines indicate lower margins of leaf and veins that were barely discernible in the negative.—Fig. 4. Radioautograph of treated leaf from plant 3 with scalded petiole. Plugging of the phloem of the petiole hindered export of radioactive phosphorus from the leaf. Movement within the leaf resulted in a thorough distribution in all veins.

Figure 3 is a radioautograph of part of the treated leaf of plant 1 and figure 4 of plant 2, both having been made at the end of the experiment. There is a much more complete distribution of radioactive phosphorus within the treated leaf in plant 2 than in plant 1, even though the same area was treated and for the same length of time in each case. The explanation is probably that in plant 1 much of the radioactive phosphorus could move directly out of the leaf via the phloem (fig. 3), whereas in plant 2 the phloem of the petiole had been plugged by scalding, so that distribution was confined to the leaf itself (fig. 4).

As was shown by radioautographs of stem sections from plants 3 and 4, large amounts of phosphorus moved back through the unscalded petiole in plant 3 and equally large amounts through the scalded petiole of plant 4, even though the plants were collected

only three hours after treatment. As table 1 reveals, approximately four times as large a surface was flooded in the treated leaf of plants 3 and 4 as in the treated leaf of plants 1 and 2. Thus, while only 10 to 15 per cent of the leaf was flooded in plants 1 and 2, 40 to 60 per cent was flooded in plants 3 and 4. These plants when treated were apparently under a considerable water deficit, as was evidenced by their partially wilted condition. In this state the whole xylem system tends to withdraw water from any possible source. In plants 1 and 2 so little of the leaf was flooded that whatever liquid entered the xylem of the veins was probably transpired within the leaf itself and consequently never moved down the petiole, via the xylem, since the transpiration stream was still moving from stem to leaf through the petiole. In plants 3 and 4, however, a much larger area of the leaf was flooded; not only did more of the solution get into the xylem of the leaf veins, but the total transpiration of the leaf was reduced. In consequence, apparently, the flooded leaf was a source of water for the rest of the plant, and the solution moved out of the leaf via the xylem of the petiole. This entire experiment was repeated, with similar results.

In other experiments, whenever a leaf vein covered by the solution was ruptured, or whenever the flooded area of the leaf was first scalded or killed with ether, radioactive phosphorus moved back through the scalded petiole even though a very small area of the leaf had been flooded. This fact indicates how much care must be exercised in treating leaves to assure export from them in the phloem only. The experiments also show that scalding the petiole does not seriously impair conductivity of its xylem elements, at least within the experimental period. The fact that the leaves did not remain wilted throughout the night, even though their petioles were scalded, whereas comparable leaves cut from the plant wilted rapidly, also indicates that the xylem was still functional despite scalding. It appears from these experiments that the downward movement of radioactive phosphorus in bean stems, reported by Bidulph (1941), may have been, contrary to his conclusions, largely in the xylem rather than in the phloem, since he cut open the veins and submerged them in a solution containing radioactive phosphorus.

If plants were kept in a saturated atmosphere before treatment, no doubt a much larger area of a leaf could be treated without danger of export via the xylem.

EXPERIMENT ON THE DIRECTION OF TRANSPORT OF RADIOACTIVE PHOSPHORUS.—Five healthy plants varying in length from 245 cm. to 360 cm. were selected, and on each plant one leaf was treated in the manner previously described. A solution containing phosphorus of approximately the same radioactivity as in the previous studies was applied. In this experiment, however, a Geiger counter was used rather than radioautographs to determine the distribution of radioactive phosphorus.

The counter was kept in a fixed position throughout, to minimize variations in the background reading. The counter was surrounded on all sides by lead and on top by a lead shield in which a slit approximately one inch long and one-half inch wide was cut directly over the counter.

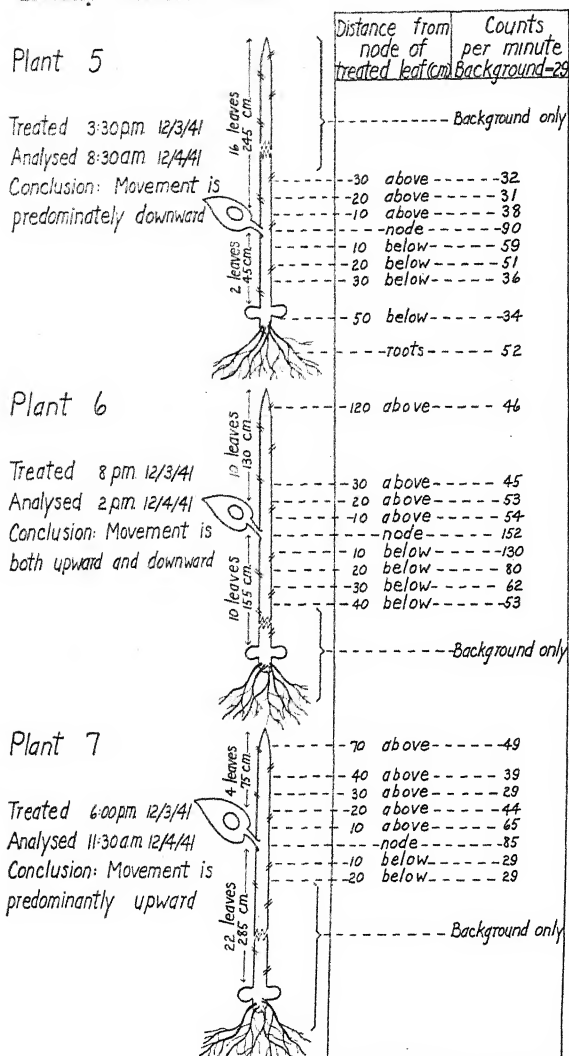


Fig. 5. Experiments on the direction of transport of radiophosphorus. Movement apparently paralleled that of foods in the plant. (Counts per minute given have not been corrected for the background count.)

Figure 5 illustrates these experiments. Each value reported is an average for ten one-minute readings made at the region of the plant indicated, by holding that portion of the intact plant directly over the slit in the lead shield. Since all portions analyzed were held in very nearly the same position with respect to the counter, the values are believed to indicate relative concentrations quite accurately.

To be certain that most of the radioactive phosphorus was exported from the leaf via the phloem rather than the xylem, two control plants were simi-

larly treated except that the petiole of the treated leaf was first scalded. One control analyzed eighteen hours after treatment, gave 37 counts per minute at the node of the treated leaf and only background values (29 counts per minute) elsewhere in the stem. The second control, analyzed nineteen hours after treatment, gave 35 counts per minute at the node of the treated leaf and only background values elsewhere in the stem.

In plant 5, as figure 5 shows, the lowermost remaining leaf was treated; and movement from it was predominantly toward the roots, which constituted the nearest sink for organic solutes. The two leaves below the treated one indicated in figure 5 were removed before the experiment. Since there was no appreciable upward movement of radiophosphorus in plant 5, even after seventeen hours, there was probably little xylem transport of radiophosphorus diffusing radially from phloem to xylem.

In plant 6 the leaf treated was near the middle of the shoot, and movement during the eighteen-hour period was both upward and downward, either because of an alternate upward and downward movement in the stem, or because of simultaneous movement in both directions.

In plant 7 (fig. 5) a leaf near the shoot tip was treated. Movement from it was predominantly toward the shoot tip, which constituted the nearest sink for organic solutes.

According to these results, movement of radioactive phosphorus, if restricted to phloem, is predominantly in the direction of food movement from the treated leaf.

EXPERIMENTS ON THE RATE OF MOVEMENT OF RADIOACTIVE PHOSPHORUS.—Figure 6 shows results of an experiment designed to study with the Geiger counter the rate of downward movement of radioactive phosphorus from a leaf on which was placed a radioactive phosphorus solution in a small plastic well.

To determine whether or not this radioactive phosphorus arrived at the base of the stem much sooner than is indicated by the Geiger counter, but in amounts too small to be detected by it, the following experiment was performed.

Six healthy squash plants were carefully selected, each between 300 and 400 cm. in length. In every case the radioactive phosphorus solution was placed on a leaf attached approximately 100 cm. below the shoot tip. The time was 6:30 P.M. One hour after treatment one of the plants was collected. Sections were cut from its stem just above the cotyledons, and radioautographs of them were made by placing the sections on aluminum foil over a piece of x-ray film, as previously described. The five remaining plants were similarly analyzed, three, six, sixteen, twenty, and twenty-four hours, respectively, after treatment. The radioautographs revealed that the plants collected sixteen, twenty, and twenty-four hours after treatment showed unmistakable evidence of radioactive phosphorus in the vascular bundles of the stem at the level of the cotyledons. In each case,

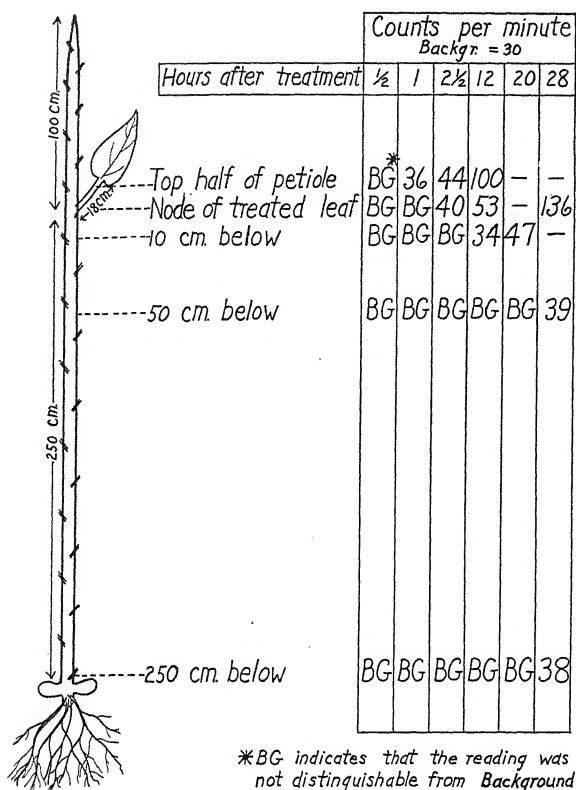


Fig. 6. Experiment on the rate of downward movement of radiophosphorus as shown by the Geiger counter.

however, readings with the Geiger counter on this portion of the stem failed to give average values different from the background reading. Apparently, then, the Geiger counter is useful in detecting only fairly large concentrations of radioactive phosphorus in a given portion of the intact plant, and therefore the rate of downward movement of radioactive phosphorus from the plant shown in figure 6 was probably much more rapid than the Geiger counter indicated.

Obviously, in the experiments just described, some time was required before an appreciable amount of the solution containing radioactive phosphorus could diffuse through the cuticle and epidermis of the leaf and come in direct contact with the mesophyll tissues and veins. A method of vacuum infiltration was devised, accordingly, whereby large quantities of the solution could be taken directly into the leaf in a very short time.

The apparatus in position, surrounding a leaf, is roughly sketched in figure 7. The 200-ml. wide-mouth bottle was three-fourths filled with an aqueous solution containing a total of approximately 150 mg. Na_2HPO_4 in which were about 5 millicuries of radioactive phosphorus.

In infiltrating the leaf, the lamina was first carefully rolled up and dipped into the bottle of solution, while the petiole was inserted into a slit in the rubber cork that plugged the top of the bottle. A hole in the cork accommodated one end of a tube that

extended directly to a suction pump attached to a water faucet. Leaks in the cork where the petiole was inserted were sealed with plasticine.

As suction was applied, air was withdrawn through the stomates of the leaf from the spongy interior and bubbled up through the solution. After about thirty seconds, suction was rapidly released, and the system allowed to return to atmospheric pressure, whereupon the solution containing radioactive phosphorus entered the leaf almost immediately to replace the air withdrawn, giving the leaf a water-logged appearance. Thus the solution came directly in contact with the veins and mesophyll cells inside the leaf. About fifteen seconds after suction was released, the bottle was removed. Usually within about fifteen to thirty minutes the excess moisture in the leaf has been absorbed or transpired so completely that the treated leaf was indistinguishable from other leaves on the plant, and was, therefore, not noticeably injured by the vacuum infiltration treatment.

That this method resulted in rapid distribution of large quantities of radioactive phosphorus throughout the plant is shown by figure 7, which

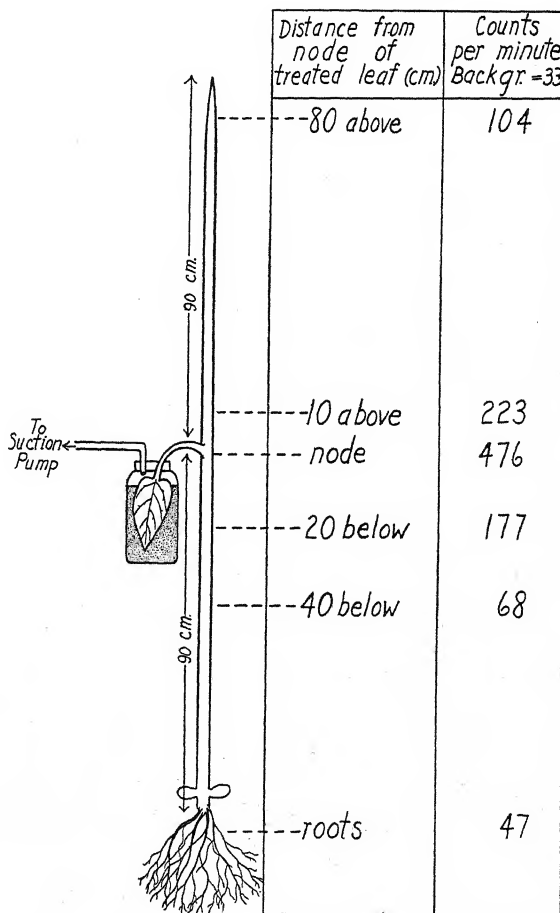


Fig. 7. Effect of vacuum infiltration on rate of phosphorus movement. Readings were made with the Geiger counter, 3 hours after infiltration.

gives readings only three hours after treatment. This plant should be compared with plant 6 of figure 5, in which a plasticine well was used.

Scalding the petiole of a leaf treated by vacuum infiltration fails to prevent this rapid movement, which, therefore, must occur largely in the xylem.

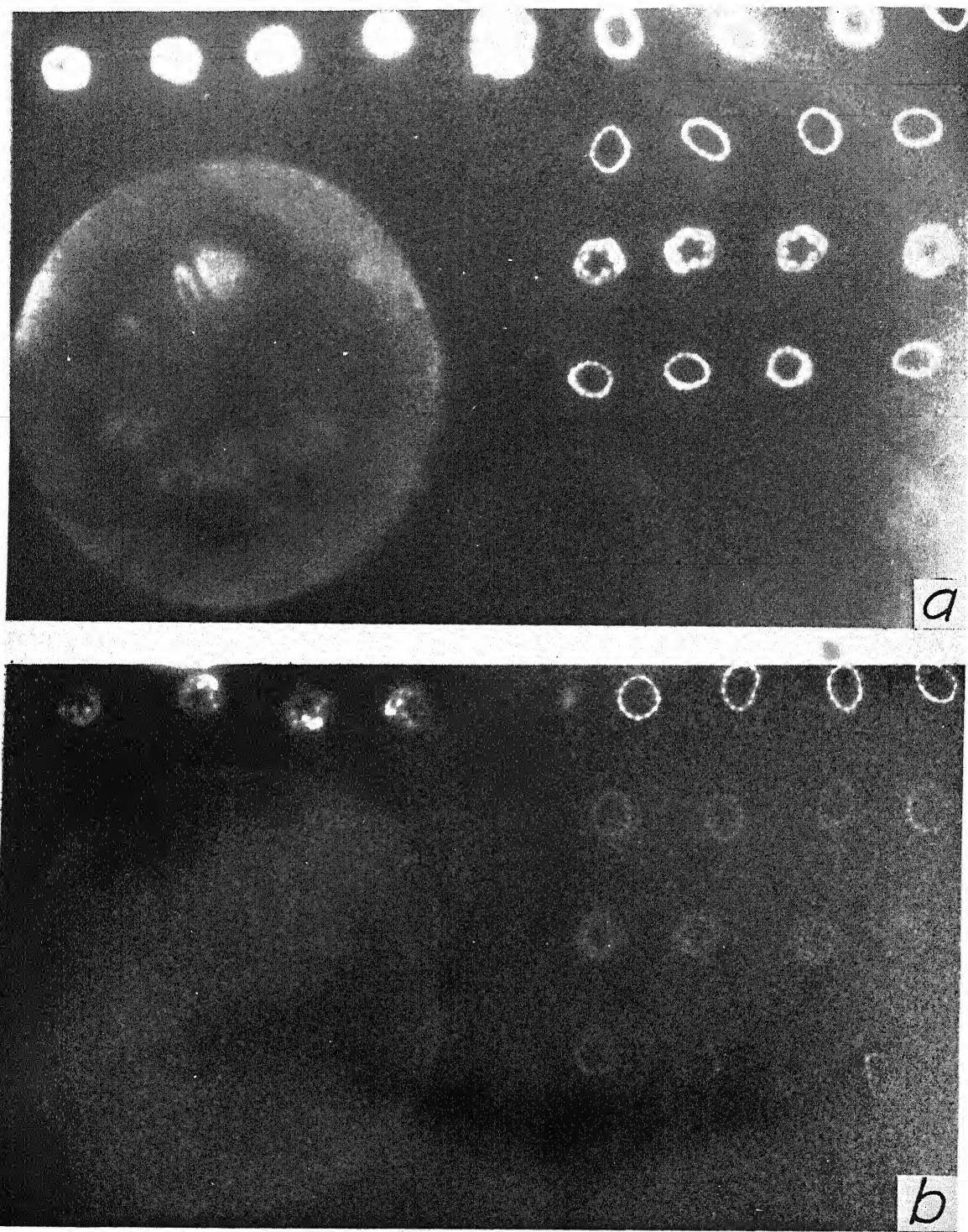


Fig. 8. Radioautographs of cross sections of fruit, stem, and petiole of squash plant.—Fig. 8a. Exposure period 12 days.—Fig. 8b. Exposure period 30 hours.

Conceivably, the movement might result from a sucking of the solution directly into the xylem elements of the hydathodes at the margin of the submerged leaf. Efforts, however, to plug the hydathodes with glue or collodion before vacuum infiltration still failed to prevent rapid xylem transport.

The following experiment offers further evidence that scalding a portion of the stem does not impair xylem transport even though sieve tubes are plugged. Beginning 20 cm. behind the tip of a squash shoot, a 10-cm. portion of the stem was scalded in boiling water for one minute. The apical 10 cm. of the shoot was then carefully immersed in a 0.1 per cent solution of eosin in the suction bottle and subjected to vacuum infiltration, after which the bottle was removed. Thirty minutes later the eosin could be traced 40 cm. below the shoot tip, having completely traversed the 10 cm. scalded portion of the stem. In a second shoot, not scalded but otherwise treated identically, the eosin could be traced 35 cm. below the shoot tip. Accordingly, vacuum infiltration treatment was abandoned as a method of studying phloem transport exclusive of xylem transport. It may, however, prove useful for such studies if the plants are placed in a saturated atmosphere before treatment, as will soon be mentioned. It is also a valuable method where redistribution or nutritional effects are being studied.

EXPERIMENTS ON THE LOCALIZATION OF RADIOACTIVE PHOSPHORUS.—Figure 8a shows a radioautograph taken of various portions of a plant on which one leaf had been treated by vacuum infiltration eighteen hours before collection. In the axil of the treated leaf was a fruit, three sections of which appear faintly in figure 8a. In the largest of these sections the radioactive phosphorus has accumulated in the seeds. In the center of the top row of sections was placed a drop of phloem exudate. That it contained radioactive phosphorus is clearly shown by the radioautograph. Sections of the stem in figure 8a show localization of phosphorus not only in the vas-

cular bundles but apparently also at the periphery of the stem, at least in certain sections. Perhaps sufficient transpiration occurred from the stem during the experiment to carry the phosphorus solution to the surface and there concentrate it. The fruit sections show a similar phenomenon. Two of the stem sections in the third row from the top in figure 8a also show a line, in the approximate position of the interfascicular cambium, which connects adjacent bundles. This line, frequently found in radioautographs of stems, is believed to result from accumulation of radioactive phosphorus in the cells of the interfascicular cambium due to their relatively high metabolic activity, even though at this stage of development of the plant these cells are not actively dividing. In future experiments, in fact, radioautographs may provide the best means to obtain indications of the relative metabolic activity of various tissues of an organ by their accumulative capacity for radioactive elements. The fact that the developing embryo within an ovule is often quite distinct in radioautographs and that the ovule itself is distinct from surrounding tissue offers further evidence for the possibility of this type of research.

A comparison of figures 8a and 8b shows how length of exposure period affects clarity and intensity of radioautographs. Serial sections of each piece of plant material to be radioautographed were cut to a uniform thickness of 200 microns, and alternate sections were used in making the two radioautographs. Identical developing and printing procedure also was followed insofar as possible. P^{32} has a half life of about two weeks. Though a saturation effect is undoubtedly involved, a greater difference in the intensity of these two radioautographs might have been anticipated; 8a received nearly ten times as long an exposure period as 8b.

The same tendency is shown by figures 9a (eleven days' exposure) and 9b (four days' exposure), similarly prepared from a plant on which one leaf had been treated with radiophosphorus solution in a

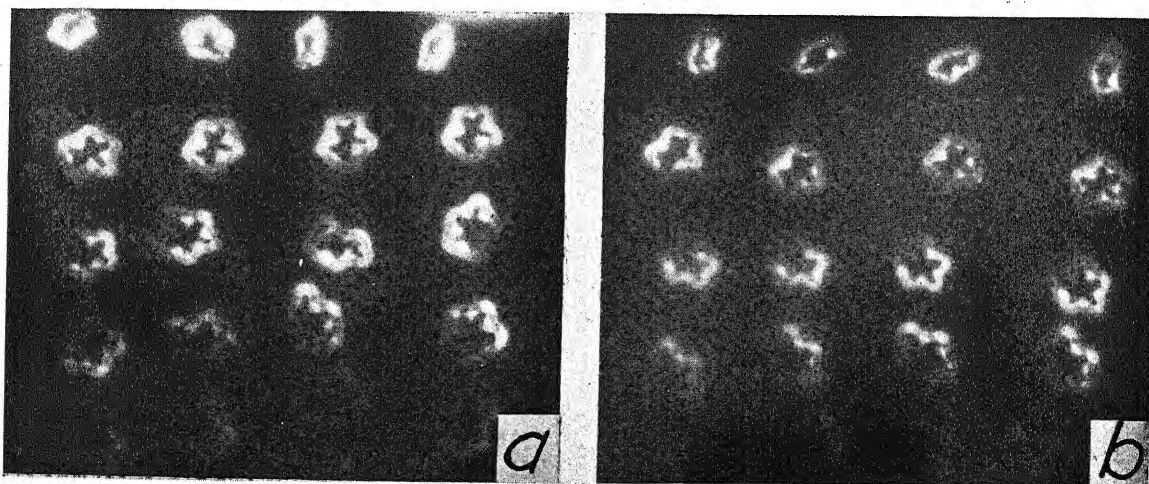


Fig. 9. Radioautographs of stem cross sections from plants injected with radiophosphorus by vacuum infiltration.—Fig. 9a. Exposure period 11 days.—Fig. 9b. Exposure period 4 days.

plasticine well, two days before collection. Both figures show tendencies for localization of P^{32} in both internal and external phloem of the bicollateral vascular bundles and in the interfascicular cambium. Here there is even less difference in intensity of the two radioautographs, though one was exposed nearly three times as long as the other. Apparently most of the exposure is effected in the first few hours following the collection. Further evidence is the fact that if some of the sections cut from a squash stem containing radiophosphorus are placed over x-ray film immediately after cutting, whereas other serial sections from it are not placed over x-ray film until twenty-four hours later, the former radioautographs are as intense after one day's exposure as are the latter after about eight days, according to the results of one experiment. The relative importance of these first few hours of exposure, though somewhat variable, is usually much greater than can be explained simply by the decreasing radioactivity in the sections. Whatever the explanation, it is desirable in making radioautographs to begin exposure as soon as practicable after collection of the treated plant, as is shown by the experiments just described.

At the time the writer left for military service several other experiments were in progress. The indications of two of these experiments will be briefly mentioned to indicate the further possibilities of this type of research. These experiments should, however, be repeated and checked more fully.

1. If a radioactive phosphorus solution is carefully placed on a small portion of a cotton leaf within a plasticine well, the radioactive phosphorus will move down the stem to, but not beyond, a ring in which the bark is removed. This fact indicates that downward movement under these circumstances must be in the phloem only. Immediately above the ring, furthermore, radioactive phosphorus is found in the xylem as well as the phloem, as shown by radioautographs. This finding would seem to demonstrate that salts can diffuse radially from phloem to xylem, a fact indicated in early work of Mason and Maskell (1928), but not widely recognized.

2. In each of four squash plants a basal leaf was treated by vacuum infiltration at the time this leaf was guttating. In two of the plants the petiole of the treated leaf had been scalded before treatment, and in each case radioautographs showed that little or no radioactive phosphorus was exported from the leaf during the three hours after treatment, although the xylem of the petiole was not injured. In the other

two plants the petiole of the treated leaf was not scalded, and in each case very large amounts of radioactive phosphorus were exported from the leaf in a comparable period, presumably through the phloem. The vacuum infiltration method may thus prove the most promising means of treating leaves with radioactive phosphorus for the purpose of studying phloem transport, provided the plants are saturated before treatment.

In experiments designed to demonstrate whether or not materials can move simultaneously in opposite directions in the phloem, radioactive phosphorus carefully applied to leaves as herein described should serve as one useful indicator.

Recently, improved methods for directing or detecting viruses, thiamin, and certain other phloem-mobile materials should permit one of these to be used as a second tracer material for studies.

Thus Hildebrand (1942) and Hildebrand and Curtis (1942) have shown that virus movement in peach seedlings can be accelerated and directed by pruning, girdling, defoliation, or shading of parts of the growing seedlings, and that even mature leaves when shaded import the virus along with foods. Bonner (1942) has restricted thiamin movement in tomato plants by steam ringing the stem. He has shown that mature leaves apparently produce thiamin and export it along a carbohydrate gradient to growing parts of the plant where it may be accumulated. He found that the thiamin requirement of *Phycomyces Blakesleeanus* provided a satisfactory method for thiamin assay in the tomato plant.

With the use of radioactive isotopes in conjunction with other tracer materials, experiments relative to the mechanism of phloem transport will no doubt soon be performed to clarify many elusive points of this fundamental problem.

SUMMARY

Methods are described whereby the rapid longitudinal movement of radioactive phosphorus can be limited to the phloem and can be studied by means of radioautographs and the Geiger counter.

Studies on the rate and direction of transport and upon the localization of radioactive phosphorus under various conditions show that when this indicator is restricted to the phloem its movement is correlated with food movement in the plant.

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CHROMOSOME NUMBER IN THE PROGENY OF TRIPLOID GLADIOLUS WITH SPECIAL REFERENCE TO THE CONTRIBUTION OF THE TRIPLOID¹

Robert E. Jones and Ronald Bamford

IN two previous reports (Bamford, 1935, 1941) concerning the chromosome number in *Gladiolus* and the relation of this factor to the hybridization of various species and varieties, one of the important objectives has been to determine the origin of the present-day commercial varieties which are tetraploids. That certain of the diploid species of this genus were concerned in the formation of these types has long been recognized (Beal, 1916); and, as McLean (1925) pointed out some time ago, certain characters in the commercial types must have come from the South African species. In terms of chromosome number, this means that certain characters of the commercial tetraploids have been derived from the South African diploid species. Whether this transfer was by means of amphidiploids, which might have been formed spontaneously after hybridization and then crossed again with other tetraploids, or whether triploids were involved, is still a question. In the first study (Bamford, 1935) it was apparent that triploids did occur among the commercial types and later it was shown (Bamford, 1941) that the probable origin of these was from either a diploid-tetraploid or the reciprocal cross, since over fifty per cent of such attempts were successful. Perhaps the most important observation in the recent study (Bamford, 1941), however, was that such triploids were partially fertile when used as the seed parent in further crosses with either of the original parents or with other diploid and tetraploid forms.

Although the thought still persists among many that triploids are sterile, an examination of the lit-

erature reveals that such is not always the case. Progeny have been produced frequently from triploids, especially when they were used as the seed parent in triploid-diploid crosses: in apple (Bergstrom, 1938; Nebel, 1933; Dermen, 1936), *Petunia* (Dermen, 1931), *Fragaria* (Yarnell, 1931), *Oenothera* (Capinpin, 1933), *Populus* (Bergstrom, 1940; Johnsson, 1940); *Tulipa* (Bamford *et al.*, 1939); Hall, 1937; Upcott and Philp, 1939), *Zea* (McClintock, 1929), *Allium* (Levan, 1933), *Datura* (Belling and Blakeslee, 1922; Satina *et al.*, 1938), *Lilium* (Satô, 1937), *Crepis* (Navashin, 1929), tomato (Lesley, 1928), potato (Perlova, 1940) and others. It has also been reported that progeny have been produced from triploid-tetraploid as well as from triploid-diploid crosses in *Petunia* (Dermen, 1931), *Phleum* (Nordenskiöld, 1941; Gregor and Sansome, 1930), and others. It would appear then that *Gladiolus* is not unusual in this respect.

Preliminary investigations (Leavenworth, 1938) showed that not only did these *Gladiolus* triploids, when used as the seed parents, behave in a fashion similar to those already reported in the literature (Bamford *et al.*, 1939; Dermen, 1936; Hall, 1937; McClintock, 1929; Navashin, 1929; Satina *et al.*, 1938; Upcott and Philp, 1939), but that some other type of chromosome behavior was taking place. Recently a similar series of observations has been made on the progeny of triploids in *Musa* (Cheesman and Dodds, 1942). This report presents the occurrence and frequency of these observations in *Gladiolus*.

MATERIALS AND METHODS.—With the exception of the previously reported triploid variety Charm (Bamford, 1935), the triploids used in this study were produced through reciprocal crosses between various tetraploid commercial varieties and diploid

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TABLE 1. *Triploid-*

Chromosome number of the progeny.....	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Chromosomes from the triploid.....	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Number of plants	2	2	0	1	2	9	9	6	3	5	6	2	19	1	4

TABLE 2. *Triploid-*

Chromosome number of the progeny.....	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
Chromosomes from the triploid.....	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Number of plants	0	0	1	1	0	2	4	4	3	1	1	1	13	0	1

TABLE 3. *Triploid-diploid and*

Chromosomes from the triploid.....	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Number of plants	2	2	1	2	2	11	13	10	6	6	7	3	32	1	5

species. Using the triploids as the female parent, seeds were obtained after crossing these with diploid species or some diploid hybrids, and also with tetraploid commercial varieties, as well as some tetraploid hybrids produced as the result of previous hybridization. Some examples illustrating the parentage of these progeny with their chromosome number are:

[Prof. Donders (60) \times *G. hirsutus* (30)] \times *G. tristis* (30)
 [*G. tristis* (30) \times Prof. Donders (60)] \times [*G. tristis* (30)
 \times *G. alatus* (30)]

[Miss Bloomington (60) \times *G. angustus* (30)] \times Dr. Moody (60)

[Prof. Donders (60) \times *G. tristis* (30)] \times [*G. primulinus* (60) \times Princeps (60)]

In all, approximately 500 plants were obtained, and of these it was possible to obtain chromosome counts from 460. It might be well to point out here that this latter group of plants is highly selective since nearly 5000 seeds were originally secured from such crosses.

All the work was done in the greenhouse during the winter months. Seeds from the triploids were gathered in the late spring and planted in pots the following fall. After one or two growing seasons or when the resulting corms were large enough, they were then planted in separate pots, and root tips were collected from each. These tips were fixed in Navashin's solution and prepared for sectioning by the short butyl alcohol method. Tissuemat was used for the embedding process, and all sections were cut 12 microns thick. Staining was accomplished by the crystal violet-iodine technique. Observations were made with the aid of a 90 \times , 1.3 apochromatic objective. All magnifications are listed with the plates.

In making the chromosome counts, it was impossible in a few cases to obtain a consistent number for some individuals. In recording these, it was necessary to list them as \pm the number which was found most frequently. The least inconsistent of these have been included in the tables, while the more variable ones were entirely eliminated from consideration.

RESULTS AND DISCUSSION.—It was presumed in all crosses that the diploid male parent contributed only fifteen chromosomes and the tetraploid male only

thirty chromosomes, because in all other crosses, such as diploid-diploid, tetraploid-diploid, and tetraploid-tetraploid, no irregularities in gametic chromosome contribution have been observed from an extensive study of the resultant hybrids.

After the chromosome number of the triploid progeny had been determined, the contribution of the triploid seed parent, when crossed with the diploid, was ascertained by subtracting the haploid number of the pollen parent. This is shown in table 1, while the contribution of the triploid, when crossed with the tetraploid as the pollen parent, is shown in table 2. In table 3 the total contribution in all cases is shown, thus combining the two previous ones, since the triploid parent was common to both types of crosses.

Throughout the course of the investigation, observations were made to determine whether certain triploids might have reacted differently when crossed with various diploids and tetraploids; but there was no indication of this in the behavior of any of the triploids in crosses with any one individual variety or species. For this reason, all triploid-diploid or triploid-tetraploid crosses were treated as a group rather than as individuals.

Triploid-diploid progeny.—It is quite obvious that there is a great variation in regard to the chromosome number (fig. 1-4) of the progeny. Plants with every complement from thirty-three to seventy-five chromosomes, with few exceptions, were observed one or more times. Despite this wide distribution and variation, it is possible to note that the majority of these plants are either triploids or tetraploids, the latter predominating.

In table 1, which shows the triploid contribution in the triploid-diploid crosses, it is obvious that the 3n/2 number of chromosomes, or approximately twenty-two to twenty-four, was contributed in only a small per cent of the cases. One might expect it to be that of greatest frequency in view of the results previously reported in some other plants. In *Tulipa* (n=12) Bamford *et al* (1939), Hall (1937), and Upcott and Philp (1939) have found that a triploid, when used as the female parent and crossed with a diploid, contributed from twelve to twenty-two chro-

diploid hybrids.

48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
6	1	3	1	7	1	3	10	8	12	20	10	63	6	12	11	2	4	2	2	1	1	1	0	0	0	0	2

tetraploid hybrids.

63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
9	3	2	2	1	4	3	6	11	8	9	8	66	4	7	13	2	5	2	0	1	1	0	0	0	1	0	0

triploid-tetraploid hybrids, combined.

33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
15	4	5	3	8	5	6	16	19	20	29	18	129	10	19	24	4	9	4	2	2	2	1	0	0	1	0	2

mosomes and in most cases from fifteen to eighteen, which would be about the $3n/2$ number. Nebel (1933) has shown in a $3n \times 2n$ cross in apple ($n=17$) that the chromosome number of the progeny ranged from thirty-five to forty-eight with the mode at 40.5, which would indicate that in most cases the triploid contribution would be somewhere near the $3n/2$ number. Capinpin (1933) in *Oenothera* ($n=7$) in a similar cross found the range to be fourteen to twenty-one with the mode at sixteen, which again approximates the $3n/2$ number from the triploid. Bergstrom (1940) and Johnsson (1940) in *Populus*, Dermen (1936) in apple and Levan (1933) in *Allium* all found similar results, with the progeny from $3n \times 2n$ crosses having somatic chromosome numbers ranging from the diploid to the triploid. In all these instances, however, practically all of the triploid progeny fell within this group. In *Gladiolus* it must be emphasized that such a contribution occurs in only a small proportion of the cases, and only a small portion of the progeny would have somatic chromosome numbers ranging from the diploid to the triploid. Within the group examined no diploids were found.

The second most obvious contribution of the triploid female in triploid-diploid hybrids of *Gladiolus* was that of thirty chromosomes, which would make the resulting progeny full triploids with regard to chromosome number. A few such progeny have been found by McClintock (1929) and Longley (1934) in *Zea*, Levan (1933) in *Allium*, and Satô (1937) in *Lilium* in crosses involving a triploid parent. Bergstrom (1940) in *Populus* and Dermen (1936) in apple, and more recently Cheesman and Dodds (1942) in *Musa*, have also found evidence of similar gametes from the triploid. Navashin (1929) in *Crepis*, in crossing a triploid F_1 *inter se* and with other sister diploid plants, found that approximately twenty-eight per cent of the progeny were triploids. This type of behavior is, therefore, not unusual in the case of triploids.

In *Gladiolus* the most common type of behavior seems to be the contribution of the full triploid number. This would mean that the progeny receiving this number would be tetraploid in nature. This is

not in general accord with the literature, although occasional tetraploid progeny from triploids have been reported. In *Solanum* (Perlova, 1940), *Tulipa* (Upcott and Philp, 1939), and *Populus* (Bergstrom, 1940; Johnsson, 1940) infrequent progeny have appeared with the full tetraploid chromosome number. However, Cheesman and Dodds (1942) in a recent report of *Musa* triploids, have emphasized that, when progeny are produced, the most vigorous ones are mostly tetraploids; that is, the triploid contribution is most frequent. This behavior in *Gladiolus* makes it possible to see that diploid characters would be transmitted to the tetraploids if this behavior has been common in the past development of commercial *Gladiolus*.

Triploid-tetraploid progeny.—Upon examining the chromosome number of the progeny (table 2) in the triploid-tetraploid crosses (fig. 5-8), it is obvious that the majority of them are either tetraploids or pentaploids. This indicates that the triploid, regardless of the pollen parent used, is consistent in the types of gametes most frequently produced. Cheesman and Dodds (1942) have expressed a comparable situation in *Musa*, "When diploid pollen is used instead of haploid, pentaploids occur in the progenies, and thirty-three chromosomes are again indicated as the most probable contribution from the female."

Twenty-four and thirty chromosome contributions have been made in these crosses in approximately the same proportions as in the triploid-diploid crosses, although the somatic chromosome number of the progeny are higher because of the thirty chromosome contribution from the pollen parent. Only a limited number of $3n \times 4n$ crosses have been previously reported; but Longley (1934) in *Zea*, Dermen (1931) in *Petunia*, and Gregor and Sansome (1930) in *Phleum* found that the triploid contributed approximately the diploid number when used in such a cross. The exception is the work of Cheesman and Dodds (1942) in *Musa*.

The full triploid somatic number was again contributed in most of the cases. This would indicate that the progeny receiving this contribution would be pentaploid. Dermen (1931) in *Petunia*, in a $3n$

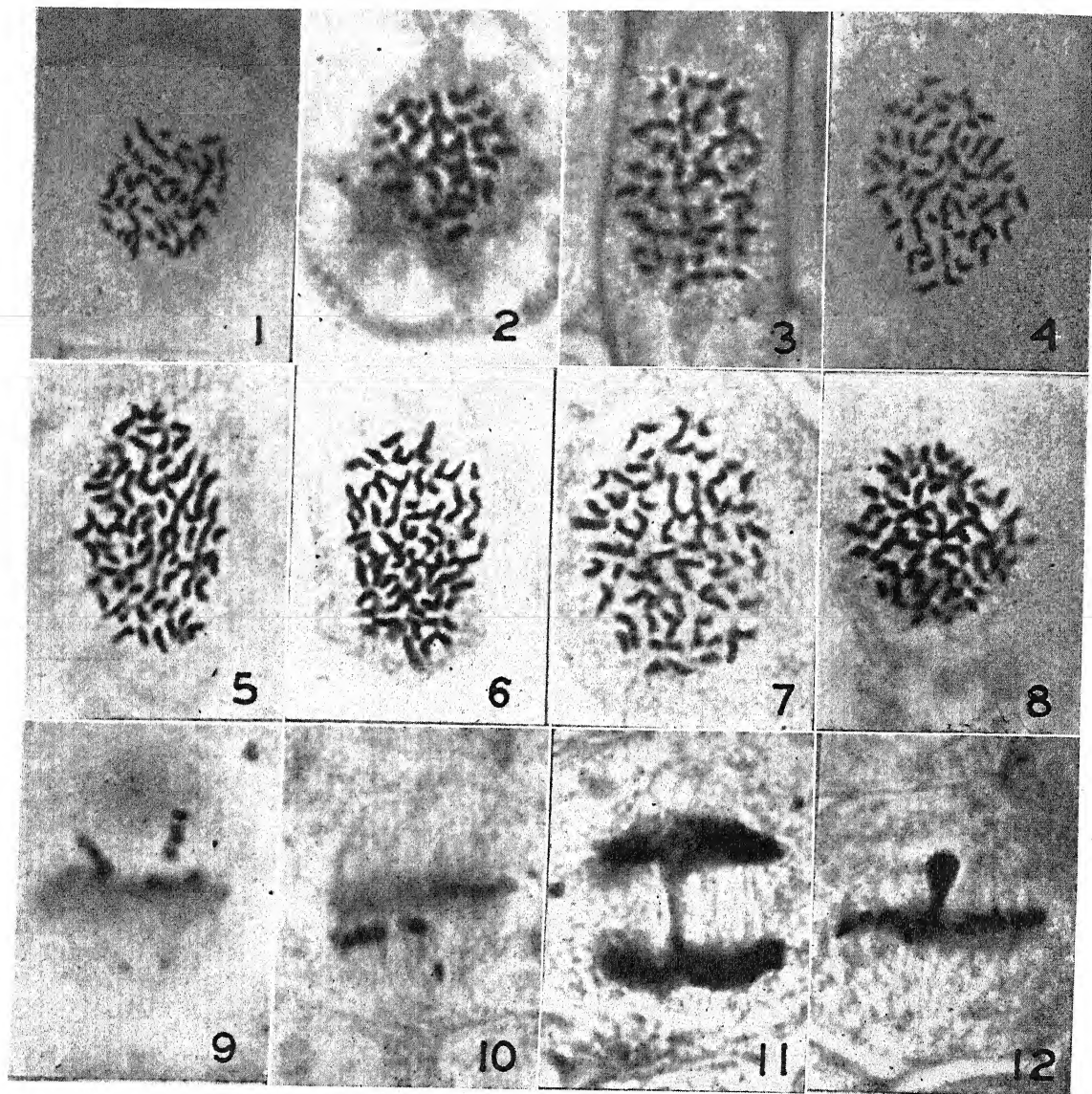


Fig. 1-12.—Fig. 1. [Joost Vondel (60) \times (*G. tristis* var. *concolor* (30) \times *G. hirsutus* (30))] \times [*G. tristis* var. *concolor* (30) \times *G. hirsutus* (30)], 42 chromosomes.—Fig. 2. [Prof. Donders (60) \times (*G. tristis* var. *concolor* (30) \times *G. callistus* (30))] \times [*G. tristis* var. *concolor* (30) \times self (30)], 48 chromosomes.—Fig. 3. [*G. tristis* var. *concolor* (30) \times Prof. Donders (60)] \times [*G. tristis* var. *concolor* (30) \times *G. alatus* (30)], 57 chromosomes.—Fig. 4. [*G. tristis* var. *concolor* (30) \times Prof. Donders (60)] \times *G. tristis* var. *concolor* (30), 60 chromosomes.—Fig. 5. [Prof. Donders (60) \times *G. undulatus* (30)] \times [Mrs. Francis King (60) \times self (60)], 75 chromosomes.—Fig. 6. [Edith Mason (60) \times *G. angustus* (30)] \times Prof. Donders (60), 77 chromosomes.—Fig. 7. [Prof. Donders (60) \times *G. angustus* (30)] \times Edith Mason (60), 78 chromosomes.—Fig. 8. [Prof. Donders (60) \times *G. tristis* var. *concolor* (30)] \times Dr. Moody (60), 78 chromosomes.—Fig. 9. Irregular mitotic division from the root tip of a triploid-tetraploid hybrid.—Fig. 10. Irregular mitotic division from the root tip of a triploid-diploid hybrid.—Fig. 11. Chromatin bridge from the root tip of a triploid-tetraploid hybrid.—Fig. 12. A persistent nucleolus found in the root tip cell of a triploid-diploid hybrid.

\times 4n cross, found one plant with the full pentaploid number of chromosomes; and Nordenskiöld (1941), using *Phleum* in such a cross, found some of the progeny to be pentaploid. In neither of these cases, however, was the full triploid contribution a general feature of such a cross. This indicates that in *Gladiolus*, the triploid, regardless of the male parent, most

generally contributes the full triploid chromosome number. That this is the most frequent type of behavior is apparent from the fact that, out of 460 progeny observed (table 2), 129 had received such a contribution.

Instances of higher chromosome contribution than forty-five are also shown. This is comparable to the

results shown by Nordenskiöld (1941) in *Phleum*.

To explain the results presented above, it is only necessary to remember that failure of pairing, unequal divisions, lagging chromosomes, and the formation of restitution nuclei have repeatedly been described as characteristic of triploids. Stout (1932) in *Heimerocallis*, Capinpin (1933) in *Oenothera*, Dermen (1936) in the Baldwin apple, Levan (1933) in *Allium*, McClintock (1929) in *Zea*, Satina and Blakeslee (1937b) in *Datura*, Satô (1937) in *Lilium tigrinum*, Belling (1924) in *Hyacinthus*, Skovsted (1933) in cotton, and many others have observed these phenomena. Although no extensive observations have been made of the embryo sac behavior in triploid *Gladiolus*, those of Westfall (1940), Bellows (1941), and Satina and Blakeslee (1937b) in other genera demonstrate that the meiotic and subsequent divisions are similar to the PMC behavior so often described, although Jensen (1938) observed that they are not apt to show such violent irregularities. One of these phenomena, or combination of them, is all that is necessary to account for the variety and extent of chromosome numbers present in the female gametes produced by the triploid. The number twenty-four could be the result of nearly normal meiosis, while the number thirty would indicate the contribution of a diploid gamete, which is not uncommon in such hybrids. The numbers eighteen to twenty-three and twenty-five to twenty-seven, as well as those from twenty-eight to twenty-nine and thirty-one to thirty-five could have resulted from lagging of chromosomes or unequal division of chromosomes during the meiotic and subsequent divisions.

It is apparent that a frequent method of behavior in triploid *Gladiolus*, in contrast to that observed in other plants, is non-reduction or the formation of a restitution nucleus. This is also borne out by Darlington (1937), who states, "Nearly all triploids that have been examined, for example, form restitution nuclei whether they are the product of hybridization of two species or of self-fertilizing a diploid." This is not an uncommon behavior because it has been observed frequently in triploids and in other hybrids by Kostoff (1932) in *Triticum*, Crane and Darlington (1927) in *Rubus*, Andersson and Gairdner (1931) in *Dianthus*, Webber (1930) in *Nicotiana*, Lawrence (1936) in *Delphinium*, Graze (1935) in *Veronica*, and many others. However, the total range from thirty-six to fifty-five is due to other phenomena in addition to the formation of restitution nuclei. Lagging and unequal division of chromosomes after non-reduction could account for gametes with chromosome numbers from thirty-six to forty-four and from forty-six to fifty-five. It is also possible that lack of pairing, with the resultant irregular behavior of univalents, might produce a gamete with greater than the diploid or triploid number of chromosomes, as has been suggested by Karpechenko (1927). When those progeny receiving the numbers thirty-six to forty-four and forty-six to fifty-five are added to the 129 which received

the full forty-five chromosomes, it shows that over fifty per cent of all the progeny fell within the group which basically have resulted from the formation of restitution nuclei.

A fact which does not show up in the tabular material is that those plants of the triploid progeny with chromosome numbers from approximately thirty-three to forty-five are weak and in most cases have never flowered. It may be that the high mortality of apparently viable seeds, young seedlings, and even young corms in the first year of growth tends to eliminate these types so that the results here presented are the product of a selective process which may not represent a true picture of all the progeny but only those which are most vigorous. In a preliminary study of a few of these plants (Leavenworth, 1938) only a small percentage of the high polyploids was observed. Again Cheesman and Dodds (1942) in *Musa* have observed this same behavior. This means, of course, that the meiotic behavior of a triploid is a determining factor, but possibly not the only one, which accounts for the high percentage of polyploids, particularly the euploids found in this study.

From all this evidence of the presence of high chromosome numbers in the functional gametes of the triploids and the subsequent selection of the resulting plants, it seems entirely possible that the characteristics of the diploids may have been transmitted to the tetraploids through the medium of hybridization which involved the formation of triploid hybrids. Navashin (1929) has spoken of "... the tendency of triploids to produce polyploid interspecific hybrids ..." during his work on *Crepis*, and Crane (Huxley, 1940) in a recent article states, "We are often unable to say how a particular polyploid arose, but it is clear that the functioning of unreduced germ cells is a frequent method and that in conjunction with hybridization it has played an important part, both in nature and in cultivation, in the origin of new forms and species." *Gladiolus*, because of its extensive cultivation and hybridization, might well have followed this pattern.

Various irregularities in somatic cell division have been observed in the above-mentioned progeny (fig. 9-12). That these have involved tissues, as well as individual cells, is obvious, because in some cases certain areas in the root tips were found to have different chromosome numbers from those of the surrounding tissues. In one instance the epidermis within a limited area gave a consistent chromosome count of eighty-eight, while part of the cortex between that and the stele contained only seventy-five chromosomes. In another case the epidermis and cortex continually differed in number throughout the observable portion of the root. There were also instances of single cells which possessed chromosome numbers well over one hundred. In addition to these irregularities, a persistent nucleolus was found in some cells. The presence of such a nucleolus has previously been reported in *Gladio-*

lus (Mensinkai, 1939). Cases of premature division of the chromosomes at metaphase also were found. In such cases some of the chromosomes preceded the others to the poles. Observations of such mitotic irregularities have been made previously by Mensinkai (1939) in *Gladiolus*, Babu (1941) in groundnut, Stout and Susa (1929) in *Hemerocallis*, and others; but no special significance seems to have been attached to the phenomena. These irregularities may indicate a certain degree of unbalance, either in chromosome number or in the rhythm of cell division.

SUMMARY

Four hundred and sixty plants were obtained from several triploid *Gladiolus* ($3n = 45$), when these were used as seed parents in crosses with diploids and tetraploids. The somatic chromosome numbers of these progeny were determined in order to find the contribution of the triploid.

The range of the somatic chromosome numbers of the progeny extended from thirty-three to eighty-eight with the majority of those resulting from the triploid-diploid cross possessing sixty chromosomes, and most of those from a triploid-tetraploid cross possessing seventy-five.

The triploids contributed nearly every chromosome complement from eighteen to sixty. The majority of the triploid progeny fell into three main groups, the largest of which received from the triploid seed parent its full chromosome complement of forty-five.

This behavior could have come about through the formation of restitution nuclei during meiosis or subsequent divisions. This type of behavior is characteristic of triploids.

Such behavior in the triploid followed by hybridization offers one explanation for the transmission of characters from the diploid species to the tetraploid forms in *Gladiolus*.

The chromosome counts listed in this study are from root tips of plants which survived at least one year of growth, and no consideration is given to the possible chromosome number of those which failed to survive. The selective process of survival may possibly have been, therefore, an additional factor of importance.

Irregularities in mitotic cell division were observed in the root tips of several of the triploid progeny.

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NICOTINE SYNTHESIS IN EXCISED TOBACCO ROOTS¹

Ray F. Dawson

EVIDENCE HAS recently accumulated which favors the view that the root organ is an important if not the sole seat of synthesis of certain of the vegetable alkaloids in those plants which produce them (Dawson, 1942; Dawson, 1942a; Krajevoj and Nechaev, 1941). This evidence has been obtained almost entirely through the use of reciprocal graft hybrids between species of different genera, some of which produce alkaloids and some of which do not. Subsequent chemical examinations then reveal the extent to which synthesis, translocation, and accumulation have occurred. The principal objection to this type of experimental approach lies in the fact that alkaloid synthesis is necessarily ascribed to the root system by inference, and that the process is not shown directly to occur in the roots as such. To complete the proof for the localization of the nicotine synthetic mechanism in the tobacco plant (Dawson, 1942a), therefore, excised roots of *Nicotiana tabacum* L. var. Turkish have been induced to grow in sterile culture so that their capacity for producing nicotine might be directly determined.

METHODS.—White (1938) has described the growth of the excised roots of two species of *Nicotiana* in sterile culture. For the purpose of the present investigation no systematic attempt has been made either to duplicate the conditions for growth prescribed by White or to ascertain the optimum environmental conditions for the growth of this particular strain of tobacco. Hence, the following description of methods of culture merely constitutes

a record of arbitrary procedures which we have followed in securing root growth sufficient to permit qualitative search for the presence of nicotine.

The basic nutrient medium possessed the following composition:

Ca(NO ₃) ₂ ·4H ₂ O	1.18	gm.
KNO ₃	0.52	gm.
MgSO ₄ ·7H ₂ O	0.46	gm.
KH ₂ PO ₄	0.136	gm.
Fe ₂ (SO ₄) ₃	8	mgm.
Sucrose	40	gm.
Extract of dried Brewer's yeast	100	ppm.
Redistilled water	2	liters

The most critical factor which we have encountered in controlling the growth of tobacco root tips under the conditions employed in this laboratory has been the temperature of the culture medium. Although no attempt has been made to determine the optimum temperature range and duration of exposure, it has been found that alternating periods of three to four days each at 24°C. and 37°C., respectively, give a rapid and sustained rate of growth. No other single factor appears to exercise so direct and immediate an influence upon the growth of these organs. Of considerable importance, also, to the growth of the roots is the presence of yeast extract. Unfortunately, the occurrence of pyridine compounds in yeast introduces a complication in studies of this type which we have been unable to avoid. The concentration of such substances in yeast, however, is so minute that the quantities of nicotine isolated

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TABLE 1. *Isolations of nicotine dipicrate from successive passages of excised tobacco roots which had been grown in sterile culture.*

Date of transfer	Date of sampling	Number of cultures	Dry weight of roots per culture	Yield of nicotine dipicrate per culture	
				Roots	External medium
			mgm.	mgm.	mgm.
1941:					
November 14	December 18	80	..	trace	0.2
December 18	
1942:					
	March 7	13	..	0.1	0.4 ^a
March 7	April 17	16	..	0.1 ^a	0.13 ^a
	June 12	11	15	0.24	0.7
	July 16	3	21	0.3	1.4
April 22	July 16	13	7	0.14	0.4
	August 3	5	10	...	0.4
June 12	August 3	25	3	...	0.1

^a Decomposition point of the unrecrystallized dipicrates was in each case 218°C. (corr.).

could not possibly have been synthesized from this source alone.²

The presence or absence of nicotine in the root tissues and spent culture solutions was established qualitatively by examination of the picrates prepared from the steam distillates of these fractions. While quantitative methods of isolation were not employed, the procedures were so standardized that the dry weights of the crystals obtained may be considered as roughly indicative of the order of magnitude of the nicotine content of the various samples. The values given in table 1, therefore, represent minimum quantities and carry only general implications regarding the total amounts of nicotine actually synthesized by the roots during the successive culture periods.

RESULTS.—Nicotine was isolated in reasonably good yields from the roots and from the residual culture medium in all successive passages extending over a total period of ten months (table 1). It is remarkable that the bulk of the alkaloid occurred in all cases in the culture medium, while only very small amounts remained in the roots. The ability of the roots to synthesize nicotine was not lost with continued subculturing, nor was there a diminution in the amounts of nicotine dipicrate isolated from these passages such as to suggest merely a dilution of the quantity of alkaloid present in the original inoculum. Furthermore, when the cultures in one passage were subdivided into groups, and these harvested at different time intervals, it was possible to observe an

² Calculations based on data obtained by Melnick and Field (1940) indicate that the order of magnitude of the nicotinic acid content of dried yeast in the quantities used in this study would have been about 0.026 to 0.032 microgram per culture. Assuming that half the nicotine molecule might have been synthesized directly from the nicotinic acid of the dried yeast only about one-thousandth or less of the nicotine synthesized in the passage of April 22 to July 16 could be accounted for in this fashion.

increase in the yield of nicotine dipicrate with increase in the length of the culture period.

DISCUSSION.—The significance of these data with respect to the problem of the origin of nicotine in the tobacco plant lies in the fact that they provide the first evidence yet obtained for the synthesis of nicotine in detached plant organs under ordinary conditions of culture. Tobacco leaves and shoots when cultured in distilled water, salt solutions, or glucose solutions invariably cease to accumulate nicotine immediately after they have been excised from the plant, and a small alkaloid loss of about 10 per cent may be expected to occur. Similarly, it has been shown (Dawson, 1942) that the callous tissue which develops on the basal ends of excised segments of tobacco stalks during culture in a moist chamber does not accumulate nicotine, although mitosis and cell enlargement have both contributed to growth in this case. On the contrary, excised tobacco roots have here been shown to synthesize and to excrete nicotine in appreciable quantities during growth.

The excretion of nicotine by excised tobacco roots under these conditions raises a question of considerable interest. If such excretion occurs from the cut ends of the excised root tips in the form of xylem or phloem exudate, then the analogy between these results and those obtained with graft hybrids would indeed be highly suggestive. In such an event it becomes possible to suggest that nicotine may be manufactured in the meristems, perhaps, of the roots and from there may be *passively* translocated in a basipetal direction through either the xylem or phloem or both. After traversing such tissues in the stem and leaf it would eventually come to be deposited in large part in the spongy and palisade parenchyma of the leaf, while the water in which it had been carried would pass out of these cells to be lost by transpiration. In such an event one would expect to find nicotine occurring in the plant body wherever water

penetrates, and the amount of nicotine in any given organ or tissue would depend upon the average rate at which water had moved into that tissue or organ in response to transpirational losses and the length of time during which this movement had been maintained. Data already published concerning the distribution of nicotine in leaves (Dawson, 1942) and in stems (Dawson, 1942a) seem to provide a basis for such speculation.

Regardless of the correctness of such an interpretation it now seems clear that, of all the organs of the tobacco plant, only the root possesses an appreciable capacity for synthesizing nicotine, and that the presence of the alkaloid in tobacco leaves in greater concentrations than occur in either stalks or roots can now be ascribed primarily to translocation and accumulation.

SUMMARY

The ability of excised tobacco root tips to synthesize nicotine when grown in sterile culture has been investigated.

In contrast to all other organs of the tobacco plant the roots were found to manufacture nicotine in appreciable amounts as growth occurred.

A PROPOSED TAXONOMIC CHANGE IN THE TRIBE MAYDEAE (FAMILY GRAMINEAE)¹

R. G. Reeves and P. C. Mangelsdorf

THE ASSIGNMENT of generic rank to *Euchlaena* Schrad. as separate from *Zea* L. was made more than a hundred years ago, as a result of the study of external characters of the ear. Since that time, *Euchlaena mexicana* Schrad. has been the subject of much study from the points of view of various branches of biology, and many important facts showing its relationship to *Zea* now may be considered as established. Most of these facts were stated or reviewed in a recent publication by Mangelsdorf and Reeves (1939), and as a full review of them would be repetitious, they will be summarized only briefly here. *E. perennis* Hitchc. in some respects shows a greater departure from *Zea* than does *E. mexicana*, and in any comparison between the two genera, *E. mexicana* serves better. The statements to follow apply to *Euchlaena mexicana* but not all of them to *E. perennis*, and for that reason special characters of *E. perennis* will be discussed in the last paragraph.

In gross morphology, neither genus is known to have a character that is lacking in the other. Distichous ears, originally thought to be absent in *Zea*, have been reported in this genus by Tavečr (1935) and Langham (1940). Paired pistillate spikelets have been found by Reeves and Mangelsdorf (unpublished) to be of fairly regular occurrence in a form of *Euchlaena*. No real difference in response to length of day can be established when the various

The bulk of the nicotine thus produced was not retained within the root tissues but was excreted to the external medium where it accumulated.

These observations complete the proof for the localization of the nicotine synthetic mechanism in the root of the tobacco plant.

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groups within the two genera are considered. Pod corn has disarticulation of the rachis and grains covered with bracts, in various degrees, in common with *Euchlaena*. Ordinarily the two genera differ in other characters but they have been believed to differ most sharply in those mentioned here.

Various morphological characters of the three genera of American Maydeae have been difficult to evaluate in the past. *Euchlaena* resembles *Tripsacum* L., a third genus, more than it does *Zea* in its disarticulating rachis, distichous spike, solitary pistillate spikelets, and covered grains. Writers of manuals have commonly subordinated these four characters to others, so as to indicate that *Euchlaena* is closely related to *Zea*; these characters have been used to separate *Euchlaena* from *Zea*. It is pertinent to ask, however, whether groups separated on the basis of these characters should necessarily be regarded as genera. It is significant that in respect to all four characters, intergrading forms between *Zea* and *Euchlaena* are known. Also, De Candolle (1882) has emphasized the error of classifying domesticated plants botanically on the basis of characters for which they are cultivated. These characters, or rather the opposite expressions of them, are precisely those for which *Zea* has been cultivated and selected.

In some regions where *Euchlaena* occurs naturally and comes into flower along with *Zea*, as in parts of Mexico, hybrid *Euchlaena*-like intergrades

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are more common than typical *Euchlaena*, and agriculturists find it necessary to renew their stock of maize every few years by securing pure seed, on account of continuous contamination. Collins (1921) observed this intergradation and accounted for the lack of the more maize-like forms in nature by their inability to survive, especially in competition with other plants. If agriculturists in these regions did not discriminate against the truly intermediate forms, or if these forms could survive in nature, *Zea* and *Euchlaena* no doubt would blend completely. Not only the intergrades occurring in these regions but probably also those reported by Taveār and Langham may be explained as hybrids, although the evidence of this in the latter two instances is less satisfactory. Assuming that this explanation is correct in all instances of intergradation between *Zea* and *Euchlaena*, opinion will vary as to whether the intergradation should be considered in taxonomic classification. For present purposes we need only to point out that numerous instances of intergradation between closely related species are known in which the intergrades have been well demonstrated to be of hybrid origin, and the *Zea-Euchlaena* intergrades agree quite satisfactorily with them. If we use any of the common criteria of species, such as distinctness or permanent isolation, we find that *Euchlaena* and *Zea* conform very well to closely related congeneric species.

The fact that hybrids can be produced between remotely classified groups like *Saccharum* and *Bambusa*, as reported by Venkatraman (1937, 1938), or like *Zea* and *Saccharum*, as reported by Janaki Ammal (1938), is of little or no importance in the *Zea-Euchlaena* problem. These remotely classified groups do not intergrade naturally, so far as reports show, and the fact that they can be hybridized artificially and even with great difficulty may indicate a fault in their present classification rather than a misconception concerning crossability in general. The necessity of interpreting crossability with caution is admitted, since certain genes, for example, may influence crossability. However, ample caution is believed to have been exercised in the present instance in respect to *Zea* and *Euchlaena*.

The two genera are as fertile, or nearly as fertile, with each other as they are with themselves, and this also applies to the F_1 and later hybrids between the genera. *Zea* and *Euchlaena* have the same chromosome number and their chromosomes are similar in morphology. Cross-over between their chromosomes is nearly normal. In so far as the gene loci have been determined, they are identical or nearly so in the two genera. The two genera are susceptible to many of the same insects and diseases. Although existing knowledge of the native range of each genus is incomplete, there is strong indication that both are natives of tropical America.

Hybridization should be mentioned again in connection with the probable origin of *Euchlaena* itself because the present writers (Mangelsdorf and Reeves, 1939) have advanced the hypothesis that

this genus originated as a hybrid between *Zea* and *Tripsacum*. Evidence in favor of this view is still being found, and nothing has been called to the writers' attention which is seriously contradictory to it. However, *Euchlaena* has not been completely demonstrated to be of hybrid origin, and there is diversity of scientific opinion on this question. The question is a peculiar one which may never lend itself to an undisputed conclusion. If *Euchlaena* should be definitely proven to be of hybrid origin, it ought still to be recognized as congeneric with *Zea*, at least if natural kinship be accepted as the main criterion. Evidence of hybrid origin is now on record for a great number of good taxonomic groups which were not formerly supposed to have originated in this manner. Phylogenetic development in general has occurred in various ways; in many groups it is convergent, in others its course is obscure. In actual practice, we find ourselves confronted with the question as to whether it is better to classify plant groups on the basis of their kinship in a broad sense, or to place the main emphasis on the mere mechanics of their origin, which is unknown for the majority of them. To make *Zea* and *Euchlaena* congeneric enables one to formulate the maximum number of prophesies and deductions and is more practical for the working taxonomist.

The close relationship between *Zea* and *Euchlaena* has been mentioned or implied in many scientific publications, only a few of which will be briefly mentioned here. Ascherson (1875) emphasized the similarity between *Euchlaena* and *Zea* and stated that *Euchlaena* appears to be merely a stunted type of *Zea*. East (1913) stated that the two groups are "simply diverse types of the same polymorphic aggregation, although they may be called species [not genera] if one desires." Many other scientific workers have implied in various ways that the difference between *Zea* and *Euchlaena* is not generic. This opinion has been expressed more recently by Langham (1938) and Beadle (1939).

Tripsacum shows very few indications of relationship to *Zea* and *Euchlaena* such as these show to each other. It can be hybridized with both *Zea* and *Euchlaena*, but this is done only with great difficulty and the hybrids are highly sterile. In these hybrids only about three pairs of chromosomes synapse and the synopsis is irregular and especially weak.

When all relevant facts known to us are considered, the relationship of *Tripsacum* to *Zea* and *Euchlaena* does not seem to be inconsistent with that shown between many other genera in the grass family. However, the relationship between *Euchlaena* and *Zea* is of a totally different rank; it is much closer and should be regarded as specific. Whether it should be considered as really specific would be an easier question to justify, but two salient facts indicate an affirmative answer: (1) The two forms are readily distinguishable by external, gross morphology. (2) Hybrid intergrades are of common occurrence only in certain localities; thus a barrier between the two forms is established.

It is therefore appropriate to propose a revision making *Zea* and *Euchlaena* congeneric. Since the name *Zea* L. was proposed prior to *Euchlaena* Schrad., the International Rules require that it be retained.

A transfer of two species into the genus *Zea* enlarges its scope, and a brief description indicating its new limits is therefore given.

ZEAL.—Staminate spikelets usually on terminal inflorescences, in sets of 2 or 3, 2-flowered, one spikelet of each set sessile, the other pedicellate; glumes membranaceous, lemma and palea hyaline. Pistillate spikelets usually on lateral inflorescences; consisting of one fertile and one sterile floret, or the latter sometimes developed; caryopses naked or covered by bracts; on a polystichous or distichous rachis in pairs, or with one member of the pair rudimentary or obsolete. Pistillate spike covered by one or several husks or spathes. Styles long, single, unbranched or slightly branched, protruding from the husks at the apex of the inflorescence. Coarse annuals or perennials. Type species *Zea mays* L.

Zea mexicana (Schrad.) comb. nov.

Synonyms:

Euchlaena mexicana Schrad. Ind. Sem. Hort. Goettingen. 1832; reprinted in Linnaea 8: Litt. 25, 1833. Collected in Mexico by Muhlenfordt.

Reana Giovanninii Brign. Ind. Sem. Hort. Mutin. 1849; quoted in Ann. Sci. Nat. III. Bot. 12: 365. 1849, in Flora n. ser. 8: 400. 1850, and in Jour. Wash. (D. C.) Acad. Sci. 12: 206. 1922. From seeds collected in Mexico and possibly also in Guatemala by Giovannini.

Euchlaena Giovanninii (Brign.) Fourn. Bull. Soc. Bot. Belg. 15: 468. 1876.

Euchlaena Bourgaei Fourn. Bull. Soc. Bot. Belg. 15: 468. 1876. Collected near Chiquihuite, Mexico.

Euchlaena luxurians (Durieu) Durieu and Aschers. Sitz.-Ber. Gesells. Naturf. Freunde Berlin. 1876: 164. 1876. Bull. Soc. Linn. Paris 1: 107. 1877.

Zea perennis (Hitchc.) comb. nov.

Synonym:

Euchlaena perennis Hitchc. Jour. Wash. (D. C.) Acad. Sci. 12: 207. 1922. Collected 1 mile south of Azpotlan (Ciudad Guzman), Jalisco, Mexico, by A. S. Hitchcock.

There is some doubt whether *Zea perennis*, a perennial, should be retained as specifically different from *Zea mexicana*, an annual, for several reasons. First, it has often been explained as a mere autotetraploid form of *Zea mexicana*, but there is little or no experimental evidence upon which to base such

an explanation. Second, it is known to be native to only one small, isolated locality. Third, it hybridizes with *Zea mexicana* very readily. However, many closely related, undisputed species hybridize with each other, and a more important question is whether the hybrid intergrades may occur in sufficient numbers to obscure the identity of the two forms. Information concerning the fertility of F_1 and subsequent generations is inconclusive, but the probability is that the sterility of such hybrids is high, since the F_1 generation would be triploid, and triploids usually show pronounced sterility. Since it has been considered to be a species until the present time and since we have insufficient reason to consider it otherwise, a proposal to change its rank to that of a variety would be premature. Such a change may be justified in the future; this depends upon the results of further studies.

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ON THE DEVELOPMENT OF XYLARY ELEMENTS IN THE FIRST INTERNODE OF *AVENA* IN DARK AND LIGHT ¹

Richard H. Goodwin

THE FIRST internode of grass seedlings has been the subject of numerous recent investigations. Of particular interest has been the extreme sensitivity of this structure to radiant energy. It has been proven that very short and weak exposures to visible light will check the elongation of this structure in completely etiolated plants. Histological studies by Avery, Burkholder and Creighton (1937) and by Araki (1939) have shown that in *Avena* both

It is well known that the formation and subsequent lignification of the secondary walls of vascular elements place certain mechanical limitations upon the potential growth of these elements and, hence, upon the tissues within which they are differentiated, at least in those cases where the tissues remain intact. It should be of interest, therefore, to investigate the nature of the relationship between vascular development and elongation in the internodes of etiolated and light-inhibited *Avena* seedlings. The results of such an analysis are presented in this paper. It is shown that radiant energy produces profound alterations in the rate of formation of vascular elements with reticulate and pitted secondary walls. The bearing of these changes on the mechanism of the light inhibition is discussed.

MATERIALS AND METHODS.—The plants used in the present study were from the same source and were grown under the same culture conditions as described in a previous paper (Goodwin, 1941). "Victory oats (from Svalöf, Sweden) were husked, and large seeds were selected. These were thoroughly wet with distilled water and then laid out, groove down, at an angle of about 60° in glass dishes on wads of absorbent cotton soaked with distilled water The dishes were placed in completely dark chambers within five minutes of wetting. The chambers, of 16 cu. ft. capacity, were air conditioned at 25° ± 0.5°C. and 80 per cent relative humidity. The air in the chambers was turned over at a rate of twice a minute and fresh air was introduced at the rate of 3 cu. ft. per minute."

Irradiations were made either with a beam of red light from a tungsten-filament Mazda ruby safe-light passed through a heat filter (a 6 cm. cell filled with ferrous ammonium sulfate, 81 g. Fe (SO₄)₂ (NH₄)₂ per 1. distilled water), the wave lengths thus obtained falling between 6000 Å and 8000 Å; or with white light from a battery of 40-watt 48-inch daylight Mazda fluorescent lamps, of which 93 per cent of the energy fell between 3800 Å and 7500 Å.

At suitable stages the plants were fixed and stored in formalin-acetic alcohol (5 cc. commercial formalin; 5 cc. glacial acetic acid; 90 cc. 50 per cent ethyl alcohol). After washing in water, most of the cortical parenchyma was removed with fine scalpels. Older plants were treated for one to four hours in Jeffrey's macerating fluid (a mixture of equal parts of 10 per cent chromic acid and 10 per cent nitric acid). The plants were then thoroughly washed and mounted in 70 per cent lactic acid which served both as a clearing agent and as a mounting medium (Simpson, 1930). Gentle pressure on the cover glass served to separate cells of the vascular bundles and to spread them out into a relatively thin layer without disturbing their positional relationships in the longitudinal direction. This method has two ad-

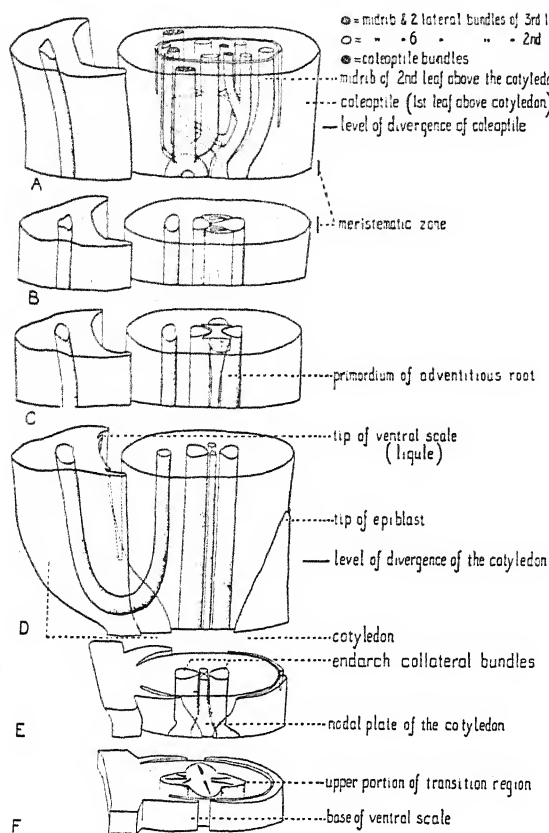


Fig. 1. The vascular anatomy of the first internode of *Avena sativa* (from Boyd and Avery, Botanical Gazette, 1936, fig. 4, reproduced through the courtesy of the University of Chicago Press).

the number and the length of the cortical parenchyma cells are reduced as a result of the light exposure. Goodwin (1941) has demonstrated that this inhibition is composed of two distinct phases: one, characterized by high sensitivity to radiant energy and correlated with the cessation of meristematic activity in the cortical parenchyma; the other, much less light-sensitive and correlated with a reduction in elongation of the cortical cells.

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vantages: first, the ease and speed with which mounts can be made; and second, the lack of damage to individual cells.

The vascular anatomy of the first internode of *Avena sativa* has been studied by Boyd and Avery (1936) and their reconstruction of the vascular system has been reproduced as figure 1. One unusual feature should be especially noted. The cotyledonary or scutellar bundle may be followed from its point of attachment to the axis at the cotyledonary or scutellar node upward, parallel to the central vascular complex, to the coleoptilar node where it bends back on itself and becomes a segment of the central vascular complex.

The nature and extent of vascular differentiation in the internodes of *Avena* seedlings of different ages grown in darkness and under two different light treatments were determined in cleared preparations. Counts were made of the numbers of the various types of tracheary elements present at three different levels in the internodes—the base (0.5 to 1 mm. above the scutellar node), the middle, and the tip (0.5 to 1 mm. below the coleoptilar node). These counts included the cells of the scutellar bundle and of the central vascular complex. The elements were grouped into the following categories depending upon the condition of their secondary walls: annular unstretched, annular stretched, spiral unstretched, spiral stretched, and pitted (including scalariform and reticulate).

Annular elements are elements which have secondary thickenings in the form of rings (fig. 2D). In the *Avena* internodes examined, these rings were found closely adjacent to one another in all elements in the earliest stages of development. Thickenings of this sort present no mechanical obstacle to stretching growth. During elongation, they may, by great expansion of the primary wall, become very widely separated (fig. 2E) before the collapse and complete rupture of the primary wall of the original cell. Elements in which the annular thickenings had been separated by a distance greater than the thickness of the rings were considered stretched.

Spiral elements are elements in which the secondary thickenings appear as spring-like spirals on the inside of the primary wall (fig. 2F). Here, as in the case of annular elements, the successive gyres of the spirals were always crowded very close together in the earliest developmental stages in all of the material examined. This type of element is also found in elongating structures, where the spiral thickenings become more or less pulled out (fig. 2G) or even perfectly straight in extreme cases. It seems probable that these spirals would tend to resist stretching growth to some extent, but even if this be true, the magnitude of such a force is unknown. Elements in which the successive gyres of the spiral thickenings were separated from one another by a distance greater than the thickness of the gyres were considered stretched. The complete array of intermediate types between annular and spiral elements (e.g. see fig. 2G) makes an accurate separation of

these two types difficult if not impossible, particularly in the cases where no stretching has taken place. Those elements which had their walls reinforced with intermittent spirals were arbitrarily classified as annular, since their walls should exhibit properties similar to those of annular elements at least where breaks occurred in the spiral thickenings.

Pitted elements are elements which have thickened secondary walls interrupted only by pits (fig. 2K). Once these secondary walls have become deposited they are no longer capable of longitudinal growth. Hence, pitted elements are not usually found in elongating structures. In the *Avena* internodes examined they were only found in regions which had completed their elongation. No evidence of tearing between these cells and the surrounding tissues was observed. Difficulty was experienced in distinguishing between pitted and unpitted elements due to the unbroken series of transitional types between annular and spiral elements on the one hand and advanced pitted types on the other (see Eames and MacDaniels, 1925, p. 92). Some of the stages in such a series are shown in figures 2 H, I, J, and 3 A, B, C, D, E. The first-formed cells of a transitional type are annular or spiral elements which show occasional narrow bands of secondary thickening connecting adjacent rings or successive gyres of a spiral (fig. 2 H and 3 A, B). Such cellulose bridges obviously impose limitations on the potential elongation of the element. For this reason walls which had an appreciable amount of bridging of this type were placed in the "pitted" category. Depending upon the extent and nature of the bridging of annular and spiral thickenings, scalariform or reticulate elements may be obtained.

RESULTS.—The results of this study will be considered under two headings: the sequence of xylary development in the internode; and the influence of light on this development.

Sequence of xylary development.—Table 1 gives a detailed sample of the basic data which are summarized in tables 2 and 3, and which are presented graphically in figures 5, 6, and 7. From an examination of these data the following conclusions may be drawn.

(1) For the first day and a half after germination annular elements are primarily differentiated. From one and a half to two and a half days, spiral elements are chiefly formed. Pitted elements are differentiated still later. This order of events is the one usually observed in the development of vascular bundles.

(2) Pitted elements are first formed at the base of the internode near the "nodal plate" of the scutellum when the plants are about two days old. A wave of differentiation surges up the internode from this basal region during the next three days. Successive stages in the development of pitted elements may be followed in a single plant in suitable vertical rows of cells. Near the scutellar node the walls may be considerably thickened, but as one passes upward, the secondary walls become thinner and thinner until the rows of tracheids or of vessel segments become

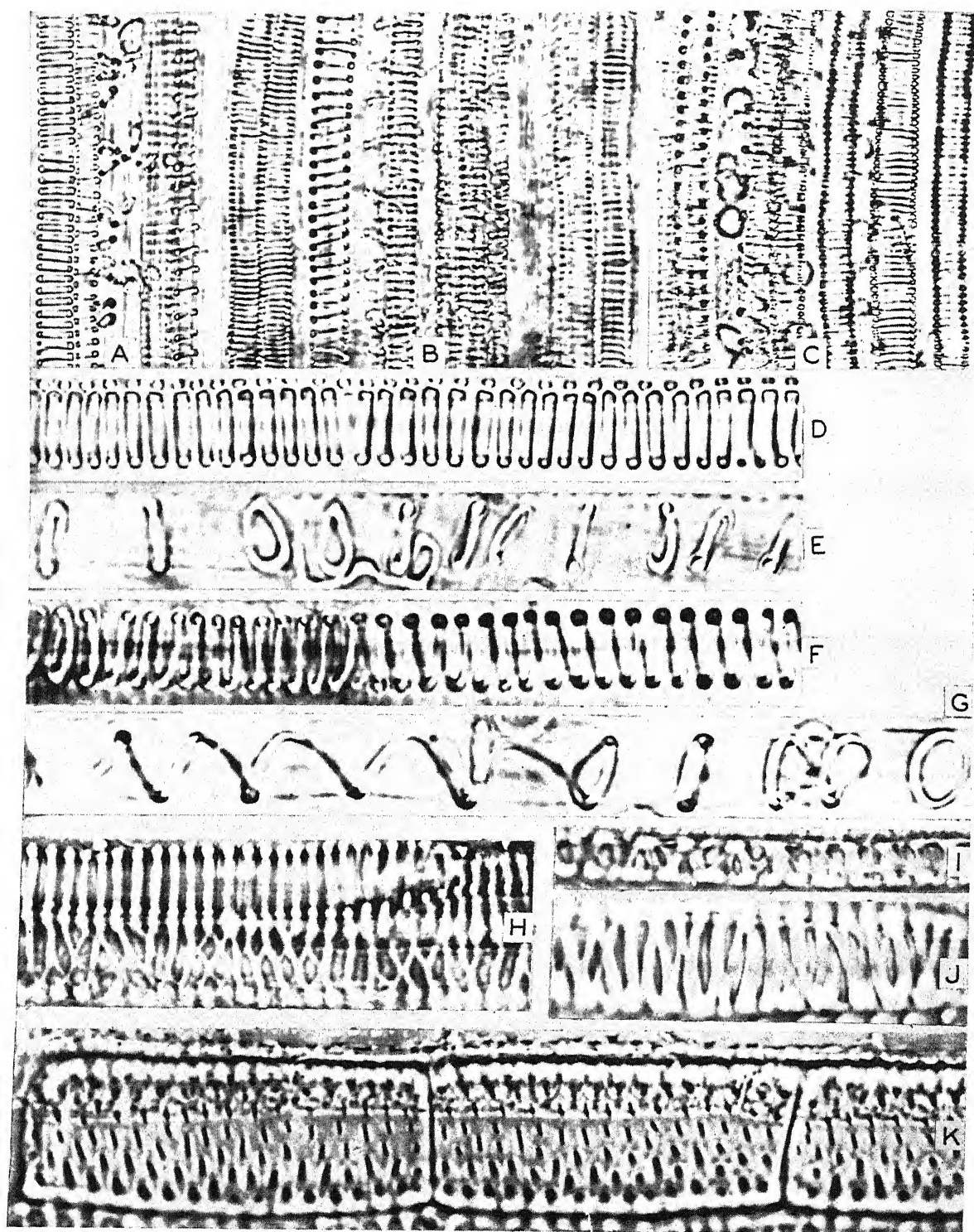


Fig. 2. Photomicrographs of xylary elements.—A–C. The central vascular complex at the weakest place near the tips of first internodes of plants sixty hours old. $\times 390$.—A. Plant grown in total darkness.—B. Plant grown in total darkness except for a five-minute exposure to red light ($0.17 \text{ erg/mm.}^2/\text{sec.}$) two days after germination.—C. Plant grown for twelve hours in white light ($4.92 \text{ ergs/mm.}^2/\text{sec.}$) after an initial dark period of two days.—D–K. Details of secondary walls. $\times 810$.—D. An annular unstretched element from the dark-grown internode shown in A.—E. An annular stretched element in the same internode.—F. A spiral unstretched element from the internode exposed to red

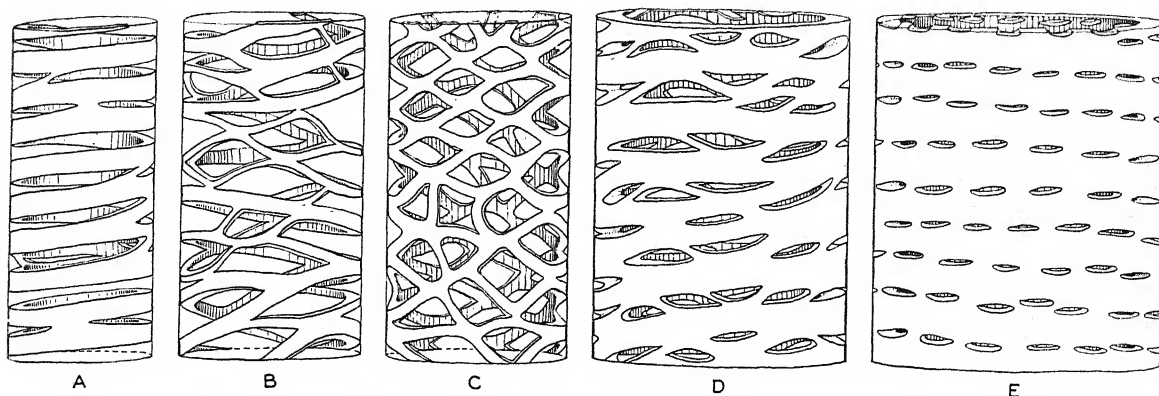


Fig. 3. Camera lucida drawings of the secondary walls of xylary elements showing transitional stages between annular, spiral and pitted types. $\times 1500$.—A-B. Elements, partly annular and partly spiral, with webbing between the rings and spirals; from a sixty-hour-old internode exposed for the last twelve hours to white light.—C-E. Pitted elements from a five-day-old internode exposed for the last three days to white light. Elements B and C give the appearance of having been somewhat pulled out during the early stages of their development.

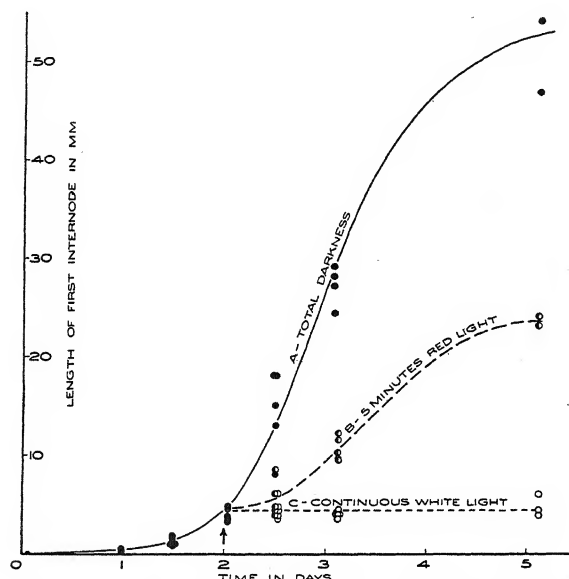


Fig. 4. Lengths of first internodes plotted against time after germination in days. Plots indicate the internode lengths of the individual plants which were used for anatomical studies.—Curve A (taken from Goodwin, 1941, fig. 1). Plants grown in total darkness (solid circles).—Curve B. Plants grown in total darkness except for a 5-minute exposure to red light ($0.17 \text{ erg/mm.}^2/\text{sec.}$) two days after germination (half open circles).—Curve C. Plants grown in continuous white light ($4.92 \text{ ergs/mm.}^2/\text{sec.}$) after an initial dark period of two days (open circles). The arrow indicates the beginning of the light exposures.

light shown in B.—G. A stretched element showing annular and spiral thickenings in the same cell; from the dark-grown internode shown in A.—H. An annular unstretched element (above) and a reticulate element (below) from the middle of the internode grown in white light, shown in C.—I. An element showing thin strips of secondary wall connecting adjacent gyres of the original spiral thickenings; from the middle of a five-day-old, dark-grown internode.—J. A scalariform element showing the anastomosing structure of the wall thickening; from a five-day-old plant grown for the last three days in white light.—K. A pitted vessel showing portions of three vessel segments; from the same internode as J. Photomicrographs were made with a $45\times$ objective and a $5.5\times$ ampliplan eyepiece on metallographic plates. The preparations were the same as those used in making the cell counts summarized in table 2, and were unstained. Photomicrographs by Mr. R. J. Maas.

rows of undifferentiated, prospective xylary elements. The formation of pitted cells takes place almost exclusively in the central vascular complex and not in the scutellar bundle as is shown in table 4.

(3) A second center of vascular differentiation is clearly in evidence by the third day at the coleoptilar node of dark-grown plants and plants irradiated with red light, whence a second wave of differentiation passes downward through the internode. This second center is not in evidence in plants grown in white light, probably because it is masked by the upward wave of differentiation which overtakes the coleoptilar node twelve hours after the beginning of the light treatment.

(4) Elongation apparently takes place almost exclusively in those portions of the internode in which no pitted elements are visibly differentiated. It has been shown (Goodwin, 1941, table 2) that the total elongation taking place in dark-grown internodes between the second and the fifth days is distributed as follows: in the basal section, none; in the middle section, 22 per cent; in the apical section, 78 per cent. Table 2 shows that at the end of two days pitted elements are present in the basal portion of the internode where no elongation occurs. After three days pitting may be found half way up. The apical portion in which the greatest elongation takes place is the last to develop elements with pitted walls. Internodes which have pitted elements throughout their length (e.g., plants grown for half a day in white light) have completed their elongation. There is some evidence that the formation of elements with webbed-spiral and reticulate thickenings may begin

just before the cessation of elongation, since a few such elements were observed which gave the impression of having been somewhat stretched or pulled out (see fig. 3B, C). This indicates that the cessation of elongation and the commencement of development of pitted elements probably coincide.

It should be mentioned that losses in numbers of annular and spiral elements in the internodes of five-day-old plants are shown by tables 2 and 3 and figures 5, 6, and 7. These are probably only apparent losses for several reasons. The excessive stretching of annular elements results in the wide separation of the annular thickenings and makes an accurate count of all of these cells impossible. Some of the spiral elements before the loss of their protoplasts are apparently converted into pitted types by a subsequent webbing of the spirals (see fig. 2F). Lastly, the large numbers of pitted elements present at these later developmental stages tend to obscure the smaller and less conspicuous annular and spiral elements.

Effect of light on development.—An examination of figure 4 and a comparison of figures 5, 6, and 7 will show in a graphic way how radiant energy inhibits the growth of etiolated internodes and at the same time modifies vascular differentiation. The pattern of development is much the same for etiolated and for irradiated plants. The difference is clearly in the rate of formation of the cells of the different types. This is brought out in table 3. Exposure to light induces a significant increase throughout the length of the internode in the number of spiral elements over dark-grown plants (see table 3, column 5; and compare fig. 2A with fig. 2B and 2C). Even more significant is the increase in number of pitted elements and their distribution. In dark-grown plants pitted elements are present only at the base of the internode after two and a half days. Plants of the same age irradiated with red light have pitted elements present more than half the way up the internode in three out of five cases; and plants irradiated with white light have pitted elements present from the base to the tip in three out of five cases and beyond the middle in all cases (see table 1). Since most of the differentiation of annular elements is completed by the beginning of the irradiations, it is not surprising that their formation is unaffected by the light exposures.

DISCUSSION.—Protoxylem and metaxylem defined.

—The present investigation has dealt chiefly with the development of the primary xylem. Plant anatomists usually make a distinction between those xylary elements first formed within a structure (protoxylem) and those matured later on (metaxylem). Since developmental processes frequently do not present sharp discontinuities, but rather grade into one another by imperceptible degrees, the precise definition of morphological terms, such as protoxylem and metaxylem, which have a developmental significance, becomes a perplexing problem. It must be remembered that even though arbitrary definitions for such terms be accepted, the morphological boundaries of the regions or structures so described

will frequently be difficult or impossible to determine (see Frey-Wyssling, 1940).

Russow (1872) first employed the term *protoxylem* to describe the first-formed xylary elements of the primary body which are usually characterized by relatively small diameter, annular or spiral thickenings, and characteristic position within the plant. The development and modifications of the concept of protoxylem and of the companion term *metaxylem* are ably discussed by Bugnon (1924), who comes to the conclusion that the only method of distinguishing between protoxylem and the later-formed metaxylem is by the larger diameter of the metaxylem elements, regardless of the nature of the thickenings of the walls. He points out, however, that in those cases in which the elements of the primary xylem are all of the same size, no such distinction can be made. The International Association of Wood Anatomists' Committee on Nomenclature (1933) has defined protoxylem as "first-formed primary xylem, with tracheary elements characterized by annular or spiral thickenings," and metaxylem as "later-formed primary xylem, with pitted tracheary elements."

Frey-Wyssling (1940) has criticized and discarded these static definitions and has emphasized that a satisfactory distinction between protoxylem and metaxylem can only be made on a developmental basis.² He defines protoxylem as primary xylem which differentiates before elongation is completed and which becomes torn (or at least somewhat stretched) during this elongation; and metaxylem as primary xylem which arises at or after the close of elongation and which remains uninjured (and unstretched) and, therefore, probably also capable of functioning.³ These terms are used in this sense in the present paper, in figures 5, 6, and 7.

According to these definitions all of the xylem differentiated in elongating regions must be protoxylem, although no morphological criteria can be used to determine the nature of newly-formed unstretched elements in fixed material. In structures which have completed their elongation, however, an examination of the primary xylem in longitudinal aspect⁴ should permit a delimitation of the protoxylem—those cells which have become stretched subsequent to the deposition of their secondary walls. At least in the *Avena* internode, the annular and spiral thickenings of very immature xylary elements, the secondary walls of which were so thin as to be only faintly visible, were always found to be crowded very close together. Hence, elements with

² See also Eames and MacDaniels (1925, pp. 89–95), and Jeffrey (1917, p. 18).

³ "Das Protoxylem ist primäres Xylem, das vor dem Streckungswachstum differenziert und während der Stengelstreckung zerrissen wird. Das Metaxylem ist primäres Xylem, das während oder nach Abschluss des Streckungswachstums entsteht; es bleibt unversehrt und somit wohl auch funktionsfähig." (Frey-Wyssling, 1940). The two phrases in parentheses are my own amplification of these definitions.

⁴ The precise protoxylem-metaxylem boundary cannot always be determined in cross section.

their annular or spiral thickenings separated to any appreciable extent were considered stretched.⁵ It should be clear, then, that on this basis protoxylem and metaxylem can be distinguished not by the types of elements present (spiral or annular), but rather by the condition of the cell walls (stretched or unstretched). Xylary elements with pitted secondary walls are incapable of undergoing stretching growth

cessation of elongation of a structure on the one hand and the development of pitted vascular elements on the other. Let us review the evidence in favor of such a correlation in the case of the first internode of *Avena*.

Internodes still capable of elongation possess at least a short growing zone in which no pitted elements are found, and conversely, all internodes

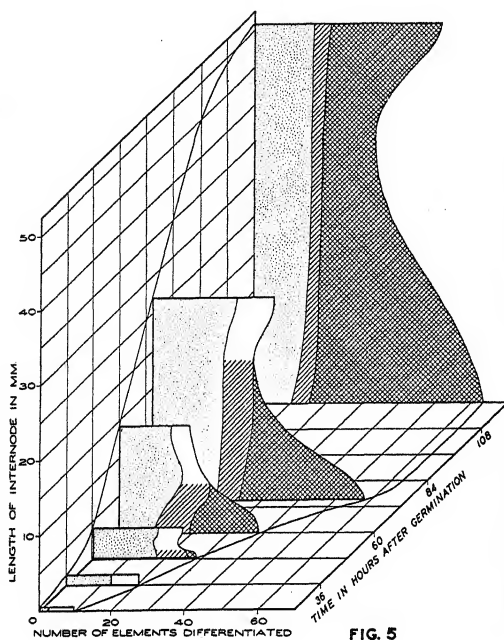


FIG. 5

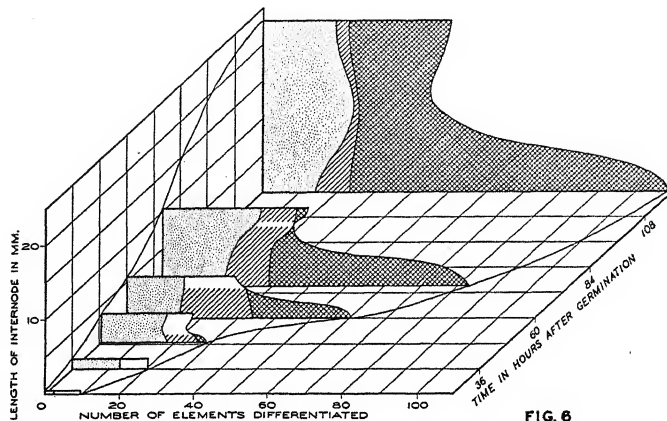


FIG. 6

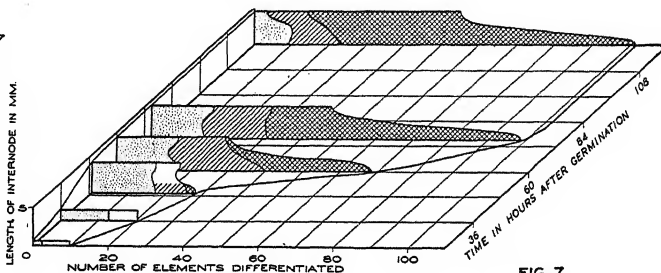


FIG. 7

Fig. 5-7. Three dimensional graphs showing the relationships between length of internode, number and type of vascular elements at various levels, and time (data from table 2). Protoxylem elements (elements with stretched secondary walls) are indicated by stippling; unpitted metaxylem elements are indicated by single cross-hatching; pitted metaxylem elements are indicated by double cross-hatching. The fate of unstretched spiral and annular elements not associated with pitted elements is undetermined. They are left unshaded since they may become stretched into protoxylem or may remain unstretched as metaxylem. For a discussion of protoxylem and metaxylem, see page 822.—Fig. 5. Plants grown in total darkness.—Fig. 6. Plants grown in total darkness except for a five-minute exposure to red light ($0.17 \text{ erg/mm.}^2/\text{sec.}$) two days after germination. Note the light inhibition of length, the increase in total number of xylary elements, the increase in number of pitted elements, and their absence from the narrow zone in which most of the subsequent elongation takes place near the tips of the seventy-four-hour-old internodes.—Fig. 7. Plants grown in continuous white light ($4.92 \text{ ergs/mm.}^2/\text{sec.}$) after an initial dark period of two days. Note the complete inhibition of elongation, the still greater increase in number of pitted elements, and their distribution throughout the entire length of the internode only twelve hours after the beginning of the light exposure.

and must, therefore, be part of the metaxylem. The presence of such elements within a structure is an indication that no further elongation is taking place.

Correlation between elongation and vascular development.—It is clear from the observations of Nathansohn (1898), Stover (1924), Frey-Wyssling (1940), and others, as well as from those described in the present paper, that a correlation, much too close to be accidental, frequently exists between the

which have a continuous sequence of pitted elements from base to tip have completed their elongation. Furthermore, pronounced increases in the extent of pitted differentiation are visible within twelve hours of the beginning of light exposures known to inhibit elongation. The first of these changes must take place in a much shorter time interval. The presence of a few somewhat stretched "pitted" elements is evidence for the simultaneous development of such elements and the cessation of stretching growth.

Nathansohn (1898) has suggested three possible ways in which the cessation of elongation and pitted secondary wall formation might be related. First,

⁵ The general usefulness of this criterion for the delimitation of protoxylem and metaxylem will depend upon whether or not stretched and unstretched elements can be readily distinguished in most other vascular plants.

pitted vascular elements, the mature lignified walls of which are incapable of elongating, might mechanically limit the elongation of the structure. Second, cessation of elongation might occur first, thereby permitting the formation of rigid secondary walls. These first two possibilities need only be considered in those cases in which a close correlation between the two processes exists. Third, the physiological condition of the structure might initiate the two processes independently and either simultaneously or at different times. If simultaneously, it becomes extremely difficult to distinguish between this and the first two possibilities; if at different times, then cessation of elongation must precede the formation of pitted elements; otherwise the pitted elements would limit growth (the first alternative mentioned above). These relationships are indicated in table 5. Nathansohn presents evidence that the first two possibilities do not occur in certain instances, and on this basis he favors the third. It does not seem safe, however, to make generalizations for all plants from the scanty data at present available.

In the case of the first internode of *Avena*, no one of these alternatives can be selected to the exclusion of the others. Indeed, the environmental conditions might determine which of them apply at any given time. A few further facts should perhaps be considered. If xylary elements actually impose a mechanical limitation on elongation, then tensions might be expected to occur between these cells and the other tissues of the organ involved. Such tensions were not observed in the first internodes of *Avena*. When the parenchyma on one side of three-day-old plants, which were exposed to white light

for the last twenty-four hours, was pared away with a razor, no bendings of the remaining internode were observed. This evidence can be advanced as an argument against the view that pitted elements mechanically limit growth, but more extensive experiments of this sort need to be done.

The recent studies of K. and M. Wuhrmann-Meyer (1939) have shown that in the *Avena* coleoptile the walls of the parenchyma cells become progressively thickened with age, and that the cellulose fibrils of such thickenings are oriented more or less parallel to the long axis of the cells. They suggest that this may be responsible for the so-called "aging" or loss of plasticity of the coleoptile. Such secondary thickening of parenchyma walls might well have a mechanical retarding influence on elongation, such as that postulated for pitted vascular elements, and might provide a mechanism for checking elongation even in structures in which no development of pitted elements occurs. The author has observed that the walls of the parenchyma cells of *Avena* internodes become considerably thickened with age and it is possible that this process may contribute toward the limitation of elongation in the internode. If so, the tensions between the cortical parenchyma and the vascular tissues mentioned in the preceding paragraph may not occur.

Auxin is a factor which has not been studied in the present investigation, but which has a bearing upon the problem of growth in length. This group of substances has been shown to increase the plasticity of the cell wall and hence to promote cell enlargement when present in suitable concentrations. As many plant organs mature, the amount of auxin decreases,

TABLE 1. The number of xylary elements differentiated at 3 different levels in first internodes of *Avena*. Each vertical column gives the data for a single plant. These data are summarized in columns 3, 4, 7 and 10 of table 2.

Age of plants		49 hours					60 hours					60 hours 5 min. red light at 49 hours					60 hours White light from 49 to 60 hours				
Light treatment		None					None														
Length of internode in mm.		3.5	3.6	3.7	4.7	5.0	8	13	15	18	18	4.0	4.5	4.7	6.1	8.5	4.0	3.7	4.5	4.9	6.1
Annular stretched	Tip	12	16	12	16	12	15	13	10	14	14	5	12	16	11	20	8	11	15	10	16
	Middle	11	19	11	13	10	21	13	13	16	14	11	14	13	11	13	6	12	15	12	14
	Base	6	18	9	13	8	14	11	5	9	7	10	12	16	10	15	8	12	16	17	11
Annular unstretched	Tip	0	7	6	2	4	5	2	5	3	3	8	7	6	5	6	1	1	3	5	6
	Middle	3	3	3	3	0	3	2	2	3	6	8	4	9	5	10	1	2	1	11	9
	Base	2	5	3	2	1	2	6	5	5	3	5	5	3	3	1	1	3	1	2	4
Spiral stretched	Tip	7	3	1	8	2	1	1	0	2	0	1	2	2	4	3	3	7	5	2	3
	Middle	6	7	7	7	1	2	3	2	3	2	5	4	4	0	0	3	2	1	1	2
	Base	5	7	7	9	5	2	3	1	1	2	1	1	1	0	6	2	3	5	5	2
Spiral unstretched	Tip	5	3	2	2	1	0	1	2	2	3	10	4	12	5	10	14	10	11	8	8
	Middle	2	3	2	3	1	1	1	2	7	4	4	10	11	12	15	19	13	14	14	12
	Base	3	6	4	12	7	4	9	5	4	4	13	18	16	10	17	17	9	14	12	4
Pitted	Tip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	3
	Middle	0	0	0	0	0	0	0	0	0	0	0	0	5	16	8	4	4	7	5	12
	Base	1	5	1	2	2	1	5	8	22	53	5	6	29	47	46	39	20	49	39	39
Total	Tip	24	29	21	28	19	21	17	17	21	20	24	25	36	25	39	29	29	34	26	36
	Middle	22	32	23	26	12	27	19	19	29	26	28	32	42	45	46	33	33	38	43	48
	Base	17	41	24	36	23	23	34	24	41	70	34	42	65	70	85	67	47	85	75	60

TABLE 2. The average number of xylary elements (\pm the standard deviation) differentiated at three different levels in the first internode of *Avena*. Each value represents an average of data from 5 different plants, except for counts taken at 123 hours when only 2 to 3 plants were examined. The final lengths of these plants are shown in figure 4.

Light treatment		Complete darkness					
Age of plants		24 hours	36 hours	49 hours ^a	60 hours ^a	75 hours	123 hours
Average length of internode in mm.		0.5	1.4	4.1	14.4	27.1	50.5
Annular stretched	Tip			13.6 \pm 2.0	13.2 \pm 1.7	19.5 \pm 3.3	13.5 \pm 0.5
	Middle	1.9 \pm 1.7	10.9 \pm 4.7	10.8 \pm 3.7	15.4 \pm 3.0	16.5 \pm 2.1	12.5 \pm 2.5
	Base			10.8 \pm 4.9	9.2 \pm 3.1	16.0 \pm 4.2	8.5 \pm 3.5
Annular unstretched	Tip			3.8 \pm 2.6	3.6 \pm 1.2	5.8 \pm 1.5	1.5 \pm 0.5
	Middle	6.9 \pm 3.1	6.6 \pm 3.5	2.4 \pm 1.2	3.2 \pm 1.5	4.8 \pm 2.5	
	Base			2.6 \pm 1.4	4.2 \pm 1.5	4.0 \pm 1.6	2.0 \pm 2.0
Spiral stretched	Tip			4.2 \pm 2.8	0.8 \pm 0.8	3.5 \pm 1.9	5.0 \pm 0.0
	Middle	0.5 \pm 0.9	1.7 \pm 1.8	5.6 \pm 2.3	2.4 \pm 0.5	3.3 \pm 2.8	3.5 \pm 1.5
	Base			6.6 \pm 1.5	1.8 \pm 0.8	2.3 \pm 1.1	2.0 \pm 1.0
Spiral unstretched	Tip			2.6 \pm 1.4	1.6 \pm 1.0	4.0 \pm 2.1	3.0 \pm 0.0
	Middle	0.6 \pm 1.1	1.1 \pm 0.8	2.2 \pm 0.8	3.0 \pm 2.3	3.8 \pm 0.9	2.5 \pm 0.5
	Base			6.4 \pm 3.2	5.2 \pm 1.9	1.8 \pm 1.5	3.0 \pm 0.0
Pitted	Tip						29.0 \pm 1.0
	Middle					3.0 \pm 2.1	19.0 \pm 2.0
	Base			2.2 \pm 1.5	17.8 \pm 19.0	33.8 \pm 9.8	47.5 \pm 11.5
Total	Tip			24.2 \pm 3.9	19.2 \pm 1.8	32.5 \pm 4.5	51.5 \pm 0.5
	Middle	9.9 \pm 4.4	20.3 \pm 4.8	23.0 \pm 6.5	24.0 \pm 4.2	31.0 \pm 0.5	37.0 \pm 3.0
	Base			28.2 \pm 8.9	38.4 \pm 17.2	57.8 \pm 11.5	63.0 \pm 16.0

Light treatment		5 minutes red light at 48 hours			White light from 48 hours on		
Age of plants		60 hours ^a	75 hours	123 hours	60 hours ^a	75 hours	123 hours
Average length of internode in mm.		5.6	10.6	23.5	4.6	4.0	4.3
Annular stretched	Tip	12.8 \pm 6.7	21.0 \pm 2.5	17.0 \pm 1.0	12.0 \pm 3.0	11.8 \pm 5.1	3.0 \pm 0.0
	Middle	12.4 \pm 1.3	15.6 \pm 4.0	19.5 \pm 1.5	11.8 \pm 3.1	11.0 \pm 3.6	8.5 \pm 5.5
	Base	12.6 \pm 2.5	14.8 \pm 2.8	12.5 \pm 1.5	12.8 \pm 3.1	12.4 \pm 3.6	6.5 \pm 4.5
Annular unstretched	Tip	6.4 \pm 1.1	5.0 \pm 2.1	3.0 \pm 0.0	3.2 \pm 2.0	5.0 \pm 1.9	3.0 \pm 3.0
	Middle	7.2 \pm 2.3	4.6 \pm 4.2	0.5 \pm 0.5	4.8 \pm 4.3	4.4 \pm 1.8	2.0 \pm 2.0
	Base	3.4 \pm 1.5	3.8 \pm 2.8	1.5 \pm 0.5	2.2 \pm 1.2	4.0 \pm 2.0	2.0 \pm 1.0
Spiral stretched	Tip	2.4 \pm 1.1	5.6 \pm 3.8	3.0 \pm 1.0	4.0 \pm 1.9	2.0 \pm 1.1	1.0 \pm 0.0
	Middle	2.6 \pm 2.2	3.8 \pm 2.3	5.0 \pm 1.0	1.8 \pm 0.8	2.0 \pm 1.1	1.5 \pm 0.5
	Base	1.8 \pm 2.1	2.2 \pm 2.2	2.5 \pm 0.5	3.4 \pm 1.4	3.6 \pm 1.1	3.0 \pm 1.0
Spiral unstretched	Tip	8.2 \pm 3.1	4.6 \pm 1.6	0.5 \pm 0.5	10.2 \pm 2.2	10.8 \pm 4.4	8.0 \pm 1.0
	Middle	10.4 \pm 3.6	5.6 \pm 3.7	0.5 \pm 0.5	14.4 \pm 2.4	12.6 \pm 3.5	6.5 \pm 1.5
	Base	14.8 \pm 2.9	8.8 \pm 2.9	7.0 \pm 3.0	11.2 \pm 4.5	12.8 \pm 4.8	10.5 \pm 2.5
Pitted	Tip		2.6 \pm 3.2 ^b	27.0 \pm 1.0	1.4 \pm 1.4	19.6 \pm 9.1	41.0 \pm 4.0
	Middle	5.8 \pm 6.0	11.4 \pm 7.1	20.0 \pm 0.0	6.4 \pm 3.0	37.8 \pm 15.8	49.0 \pm 3.0
	Base	26.6 \pm 18.4	51.4 \pm 6.5	84.5 \pm 2.5	37.2 \pm 9.4	65.0 \pm 20.2	78.0 \pm 9.0
Total	Tip	29.8 \pm 6.4	38.8 \pm 7.3	50.5 \pm 0.5	30.8 \pm 3.7	49.2 \pm 7.6	56.0 \pm 8.0
	Middle	38.6 \pm 7.3	41.0 \pm 8.2	45.5 \pm 0.5	39.0 \pm 5.8	67.8 \pm 18.8	67.5 \pm 6.5
	Base	59.2 \pm 18.7	81.0 \pm 12.5	108.0 \pm 3.0	66.8 \pm 12.9	97.8 \pm 21.2	100.0 \pm 18.0

^a Data for individual plants are given in table 1.

^b Only two of these plants had pitted elements within one mm. of the tip of the internode. In these cases no pitted elements were present at a distance of two mm. from the tip. It will be noticed that the internodes of these plants could have been expected approximately to double their length.

TABLE 3. The average number of annular, spiral and pitted xylary elements in the internodes of dark-grown plants compared with that of plants irradiated with red and white light. Data are taken from table 2.

			Number of elements differentiated at:				Number of elements differentiated between:		
			49 hours	60 hours	75 hours	123 hours	49 & 60 hours	60 & 75 hours	75 & 123 hours
Total annular elements	Dark-grown plants	Tip	17.4	16.8	25.3	15.0	— 0.6	+ 8.5	—10.3
		Middle	13.2	18.6	21.3	12.5	+ 5.4	+ 2.7	— 8.8
		Base	13.4	13.4	20.0	10.5	0.0	+ 6.6	— 9.5
	5-min. red light	Tip	17.4	19.2	26.0	20.0	+ 1.8	+ 6.8	— 6.0
		Middle	13.2	19.6	19.6	20.0	+ 6.4	+ 0.6	— 0.2
		Base	13.4	16.0	16.0	14.0	+ 2.6	+ 2.6	— 4.6
	Constant white light	Tip	17.4	15.2	16.8	6.0	— 2.2	+ 1.6	—10.8
		Middle	13.2	16.6	15.4	10.5	+ 3.4	— 1.2	— 4.9
		Base	13.4	15.0	16.4	8.5	+ 1.6	+ 1.4	— 7.9
Total spiral elements	Dark-grown plants	Tip	6.8	2.4	7.5	8.0	— 4.4	+ 5.1	+ 0.5
		Middle	7.8	5.4	7.1	6.0	— 2.4	+ 1.7	— 1.1
		Base	13.0	7.0	4.1	5.0	— 6.0	— 2.9	+ 0.9
	5-min. red light	Tip	6.8	10.6	10.2	3.5	+ 3.8	— 0.4	— 6.7
		Middle	7.8	13.0	9.4	5.5	+ 5.2	— 3.6	— 3.9
		Base	13.0	16.6	11.0	9.5	+ 3.6	— 5.6	— 1.5
	Constant white light	Tip	6.8	14.2	12.8	9.0	+ 7.4	— 1.4	— 3.8
		Middle	7.8	16.2	14.6	8.0	+ 8.4	— 1.6	— 6.6
		Base	13.0	14.6	16.4	13.5	+ 1.6	+ 1.8	— 2.9
Pitted elements	Dark-grown plants	Tip	0.0	0.0	0.0	29.0	0.0	0.0	+29.0
		Middle	0.0	0.0	3.0	19.0	0.0	+ 3.0	+16.0
		Base	2.2	17.8	33.8	47.5	+15.6	+16.0	+13.7
	5-min. red light	Tip	0.0	0.0	2.6	27.0	0.0	+ 2.6	+24.4
		Middle	0.0	5.8	11.4	20.0	+ 5.8	+ 5.6	+ 8.6
		Base	2.2	26.6	51.4	84.5	+24.4	+24.8	+33.1
	Constant white light	Tip	0.0	1.4	19.6	41.0	+ 1.4	+18.2	+21.4
		Middle	0.0	6.4	37.8	49.0	+ 6.4	+31.4	+11.2
		Base	2.2	37.2	65.0	78.0	+35.0	+27.8	+13.0

and this may produce a physiological condition unfavorable to growth even before the cell walls have become appreciably thickened.

Numerous previous physiological investigations on the growth of the first internode of *Avena* have completely disregarded the vascular system. In the present study the close correlation between cessation of elongation and the development of pitted xylary elements has been emphasized. In the writer's opinion the possible rôle of the xylem in limiting elongation should be given serious consideration and further study.

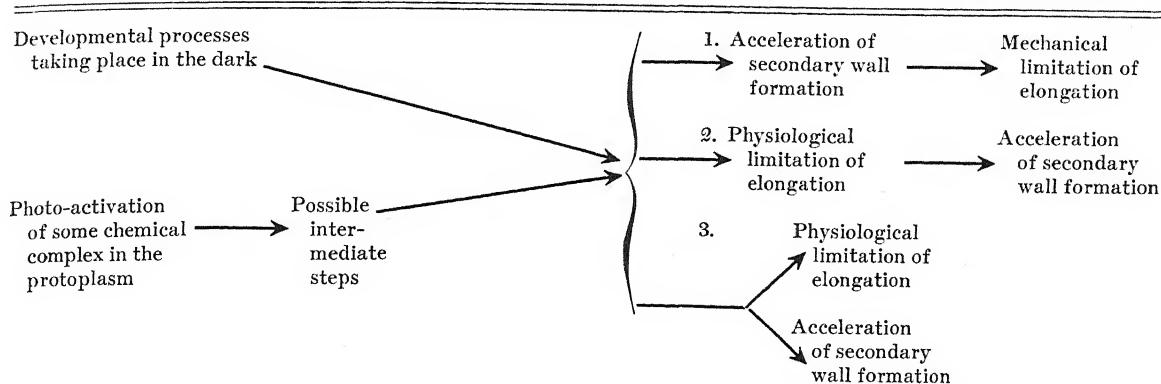
Effect of light on vascular development.—The retarding influence of light on the elongation of plants is too well known to require any amplification here. Little has been published, however, on the influence of light on the vascular system. The recent work of Smith and Kersten (1941, 1942) has shown that soft x-radiation will bring about a strong inhibition of the elongation of *Vicia* roots; and that this inhibition is closely correlated with the forma-

tion of pitted elements in the procambial strands much closer to the apical meristem than is normally

TABLE 4. The number of pitted and unpitted xylary elements in the scutellar bundle and in the central vascular complex of five-day-old internodes.

		Scutellar bundle		Central vascular complex	
		Unpitted cells	Pitted cells	Unpitted cells	Pitted cells
Dark-grown plants	Tip	13	0	10	28
	Middle	11	0	8	21
	Base	13	2	7	57
5-min. red light	Tip	15	0	8	27
	Middle	15	0	11	20
	Base	15	0	9	84
Constant white light	Tip	14	0	5	45
	Middle	15	2	13	44
	Base	17	1	14	86

TABLE 5. Diagram indicating possible relationship between light, elongation and secondary wall formation.



the case. In addition, other abnormal anatomical features are induced by this rather drastic treatment.

The visible irradiations used in the present investigation, on the other hand, are similar to those experienced by seedlings in the course of their natural development, and yet their effect in accelerating vascular differentiation and particularly the development of pitted elements is very marked. Whether the same phenomena are in operation here as in the case reported by Smith and Kersten is not known. The bearing of pitted secondary walls on the problem of elongation has already been discussed. The relation between growth in length and the inhibition of this process by light is obvious.

K. and M. Wuhrmann-Meyer (1939) have demonstrated that the secondary thickening of the parenchyma walls of *Avena* coleoptiles is hastened by light exposures which retard the elongation of this structure. This appears to be a developmental response very similar to that exhibited by the vascular elements of the *Avena* internode, and it is probable that the parenchyma of the internode is also affected in the same way.

The relationship between radiant energy and the two developmental processes under consideration is indicated in table 5. Secondary wall formation and the ultimate cessation of elongation both occur in complete darkness, but both of these processes are accelerated by exposure to radiant energy. The mechanism of this acceleration remains obscure.

In a previous paper (Goodwin, 1941) it was pointed out that if growth inhibitions of the first

internode at the lowest light intensities were due to inactivation of auxin by light, a very high efficiency in the utilization of the radiant energy would have to be postulated. It was concluded that some other mechanism was more probable at least at these low intensities although the possible rôle of auxin was by no means eliminated by this line of reasoning, particularly at higher light intensities.

SUMMARY

A study has been made of the development of vascular elements in the first internodes of *Avena* seedlings. Annular, spiral, and pitted elements are successively differentiated, and the presence of transitional types is emphasized. The first center of development of pitted elements arises at the scutellar node, and a wave of differentiation surges upward from this point through the internode. A second center occurs later at the coleoptilar node.

The rate of formation of spiral and particularly of pitted elements is greatly increased by exposures to visible light, pronounced effects being observed as early as twelve hours following weak irradiations. The close correlation between the light inhibition of elongation of the first internode and the formation of pitted xylary elements which are incapable of growth in length is brought out. The possible rôle of the xylem as an important cog in the light inhibition mechanism is discussed.

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SPECIES DIFFERENCES WITH RESPECT TO WATER ABSORPTION AT LOW SOIL TEMPERATURES¹

Paul J. Kramer

IT WOULD be expected that plants native to warm climates and normally growing in warm soils would suffer a greater reduction in water intake when the soil is cooled than plants which normally grow in cooler soils. This opinion is supported by the results of one of the earliest investigations of the effects of cold soil on water absorption, that of Sachs (1875). He found that, under conditions of low transpiration, gourd and tobacco plants wilted at soil temperatures of 3° to 5°C., but they recovered when the soil was warmed to 12° or 18°C. Cabbage and turnips, on the contrary, remained unwilted even when the soil was cooled almost to freezing. Although a number of studies of the effects of cold soil on water absorption of individual species have been made, few direct comparisons of species, especially species from northern and southern habitats are available. The most extensive investigation is that of Döring (1935) who observed the reaction of fifty-seven species of European plants from various habitats and concluded that no clear relation exists between the distribution of plants and the effects of low temperature on their water absorption. Inspection of Döring's data indicates, however, that water intake by plants from northern flat moors and high moors was reduced less by cooling the soil than was water intake of forest trees and plants from warmer drier soils. No review of the literature will be made because the earlier work has been previously cited (Kramer, 1940) and the more recent investigations will be discussed later in this article.

This paper reports the results of an investigation of the effects of low temperatures on water absorption by several species not previously studied. Part of the experiments were specially planned to compare the behavior of species which normally grow in warm soil with that of species which normally grow at least part of the season in cold soil. Among the species compared were cotton (*Gossypium*

hirsutum L.) and watermelon (*Citrullus vulgaris* Schrad.), which thrive only in warm soil, and Georgia collards (*Brassica oleracea* var. *acephala* DC), which grow out-of-doors all winter and survive temperatures far below freezing. Among the woody species compared were loblolly pine (*Pinus taeda* L.) and slash pine (*P. caribaea* Morelet), which are natives of the southeastern part of the United States, and red pine (*P. resinosa* Ait.) and white pine (*P. strobus* L.), which have a more northern distribution. Other species not directly compared but for which data were obtained were privet (*Ligustrum japonicum* Thunb.), American elm (*Ulmus americana* L.) and sunflower (*Helianthus annuus* L.).

METHODS.—The herbaceous species were grown from seed in four or five quart metal containers while the woody species were transplanted into similar containers. The herbaceous species were usually about two months old and fifteen to eighteen inches high when used in experiments. The woody species were of about the same height, but of course much older, the loblolly and slash pine being over a year old and the red and white pine four or five years old. The elms were year-old seedlings, and the privets were from cuttings which were probably four or five years old. The pines had been out-of-doors during the winter prior to the time of the experiments.

The tops of the containers were covered with oil cloth during the actual experiments to prevent evaporation from the soil surface. The experiments were performed in two adjacent water baths located in a sunny part of the greenhouse. Six or eight containers were placed in each bath and kept at 25°C. for four or five days. The containers were weighed daily and the water lost by transpiration was replaced. Having established the relative transpiration rates of the groups in the two baths while both were at 25°C. the temperature in one bath was lowered about 5°C. each night while the temperature of the

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other or control bath was always kept at 25°C. The rate of transpiration of the cooled group was expressed as a percentage of the rate of the control group kept at 25°C. If the original ratio of cooled-control was not 100 per cent, each of the ratios obtained during the period of cooling was divided by the average ratio obtained during the four- or five-day period before cooling. For example, suppose the ratio of cooled: uncooled averaged .85 for the four- or five-day preliminary period during which both groups were kept at 25°C. As the soil temperature was then lowered, the ratios decreased to .60, .50, .20 and .10. Each of these ratios was divided by .85. In another experiment the ratio of cooled: uncooled might be 1.10. Then all ratios obtained during the cooling process would be divided by 1.10. This corrected all ratios to the same percentage basis, so that various species could be compared on the same graph. No control of the environment other than soil temperature was possible, but since the tops of all plants in any particular experiment were always exposed to the same environment any difference in transpiration between plants in cooled and uncooled soil may be assumed to result from decreased absorption in the cooled soil. This procedure is based on the assumption that absorption is approximately equal to transpiration. Such an assumption is not strictly true for short periods of time, especially if transpiration is rapid, but it is so nearly true for twenty-four-hour periods that the error is unimportant.

This method has certain limitations. It cannot be used on plants which are rapidly increasing in leaf area because low soil temperatures reduce the rate of growth of the cooled group as compared to the uncooled group, changing their relative leaf areas and rates of transpiration. The full effect of cooling the soil can only be observed when atmospheric conditions are favorable for high transpiration, so that

reliable data can only be obtained on sunny days. It was observed that the transpiration ratio cooled: uncooled was much higher on cloudy days than on bright days, indicating that the potential rate of transpiration does not exceed the rate of absorption from cold soil as much on cloudy days as on clear days. Bialoglowski (1936) observed that cooling the soil did not reduce night transpiration of lemons because the night rate at 25°C. was already lower than the day rate at freezing; hence the rate of absorption never became a limiting factor. The advantages of this method are that it eliminates the need for expensive environmental control apparatus and makes it unnecessary to pair the cooled and uncooled groups so that their transpiration rates are equal. This method also makes determinations of leaf areas unnecessary, saving labor, especially with conifers, and obviating the defoliation of the experimental plants at the conclusion of an experiment.

RESULTS AND DISCUSSION.—The data are summarized in table 1 and in figures 1 and 2. They are grouped in the table to show the actual comparisons made. The first three experiments were comparisons of collards and cotton, the fourth experiment was a comparison of collards with watermelon, while the fifth experiment was made on watermelon alone. All four species of pine were run simultaneously and the experiment was then repeated. Sunflower, elm, and privet were studied separately.

The most important data are those obtained by direct comparison of species. Inspection of table 1 and figure 1 shows that the behavior of Georgia collards is very different from that of cotton and watermelon. This difference appeared at a soil temperature of 15°C., and at 10°C. collards were absorbing about four times as much water as cotton and watermelons. Table 1 and figure 2 show that there is also a difference in the behavior of the northern and

TABLE 1. *Effects of soil temperature on transpiration.*

Expt.	Material	Date	No. of plants per exp.	Final soil temp.	Transpiration of cooled plants as % of controls at 25°C.
1	Collards	June, 1939	6	12°C.	63.0
	Cotton	June, 1939	6	12	7.4
2	Collards	June, 1939	6	11	79.0
	Cotton	June, 1939	6	11	22.0
3	Collards	June, 1939	6	4.3	53.0
	Cotton	June, 1939	6	4.3	4.3
4	Collards	Aug., 1940	6	1.0	33.0
	Watermelon	Aug., 1940	6	1.0	1.4
5	Watermelon	July, 1940	12	6.5	9.0
6	Loblolly pine	Apr., 1942	4	0.5	13.7
	Slash pine	Apr., 1942	4	0.5	13.9
	White pine	Apr., 1942	4	0.5	37.7
	Red pine	Apr., 1942	4	0.5	25.0
7	Elm	Sept., 1935	14	0.5	25.0
8	Privet	Aug., 1935	12	2.5	47.0
9	Sunflower	June, 1936	12	1.0	27.0

southern species of pine. The absorption of water is reduced much more in the temperature range from 15°C. to freezing in loblolly and slash pine than in red and white pine which have more northern ranges. Kozłowski (1941) also found that water intake of loblolly pine was reduced more than that of white pine by cooling the soil, the difference being greatest at 5° to 10°C. Cooling the soil reduced water absorption of the broad-leaf evergreen privet less than absorption of the deciduous species, American elm. The reduction in absorption of sunflower is intermediate in magnitude between that of collards and that of cotton and watermelon, and within the

range of results reported by Clements and Martin (1934) for sunflower.

In general these data indicate some correlation between the reaction of a species to cooling of the soil and the temperature of the soil in which the species normally grows. Cotton and watermelons thrive only in warm soil while collards survive soil temperatures below freezing. All of the woody species studied, except possibly slash pine, grow in soils which are often frozen, at least at the surface, but the northern species have their roots in soil which is usually somewhat cooler than that in which the southern species grow.

These results support the work of other investigators. Duncan and Cooke (1932) reported that as the temperature was lowered from 28°C. to 10°C. the absorption of water by sugar cane plants was progressively decreased. Bialogłowski (1936) found that the day transpiration rate of lemon cuttings growing in nutrient solution in a constant environment chamber decreased as the solution was cooled below 25°C. or raised above 35°C. The transpiration rate of plants kept at 5°C. for two days was only 18 per cent of the rate at 25°C. Haas (1936) reported that the transpiration rate of lemon cuttings growing in soil increased as the temperature was increased from 19° to 31°C., but decreased at 35°C. The transpiration of grapefruit cuttings increased with increasing temperature from 19° to 27°C., but decreased from 31° to 35°C. Apparently such moderate soil temperatures as 15° to 20°C. measurably decrease water intake of citrus trees. Arndt (1937) found that cotton growing in a greenhouse wilted at soil temperatures of 17° to 20°C. and at solution culture temperatures of 10° to 18°C. The exact temperature of wilting seemed to depend on the rate of transpiration, wilting occurring at higher temperature when transpiration was rapid than when it was slow.

Brown (1939) found that water absorption by Bermuda grass was sufficiently retarded by cooling the soil to 10°C. to cause wilting, while Canada and Kentucky bluegrass were unaffected at this temperature. Schroeder (1939) discovered that extensive injury to the fall crop of greenhouse cucumbers is caused by a deficiency of water resulting from cold soil. Cucumbers apparently require a soil temperature higher than 20°C. to insure adequate water absorption and vigorous growth.

The data given in figure 1 and table 1 and the investigations just cited emphasize the fact that serious reduction in water intake is not limited to temperatures near freezing, but may occur in some species at temperatures between 10° and 20°C. It will be noted from figure 1 that watermelon absorbed only half as much water at 15°C. as at 25°C. Reduction in water intake at comparatively high temperatures was also reported for citrus fruits, cotton, cucumbers and sugar cane in the papers previously cited. Soil temperature has long been considered a factor of considerable ecological importance. It is now very properly receiving increasing attention as

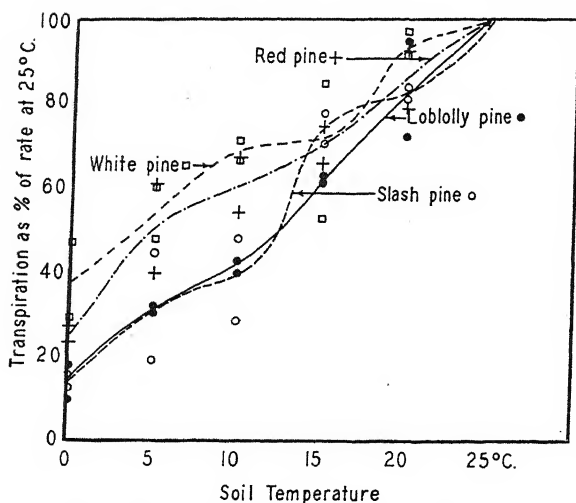
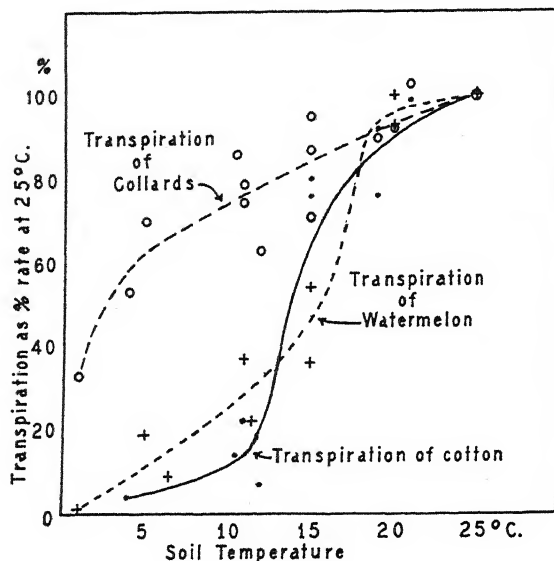


Fig. 1-2.—Fig. 1 (above). Effects of soil temperature on absorption and transpiration of collards, cotton and watermelons.—Fig. 2 (below). Effects of soil temperature on absorption and transpiration of loblolly, slash, red, and white pine.

an important factor in the growth of cultivated plants both under field conditions and in greenhouses. Much winter injury is really injury from desiccation brought about by sun and wind causing excessive transpiration at times when the soil is frozen or so cold that absorption is too slow to replace the water lost in transpiration. Even the cooling of the soil resulting from a period of near-freezing weather or from watering greenhouse beds with cold water (Schroeder, 1939) may be sufficient to produce injury.

Not only the absorption of water, but the absorption of mineral salts and various internal physiological processes, are affected by soil temperature. Jones (1938) found that a troublesome chlorosis of gardenias occurs at soil temperatures of 18°C. or lower and disappears in warm soil. Furthermore, the rate of growth, size of leaves, and reproductive cycle of the plants were affected by soil temperature. Nightingale and Blake (1934) reported that Stayman Winesap apple trees in sand cultures at 45°F. were unable to translocate nitrogen from roots to shoots during a two-weeks experimental period. Batjer, Magness and Regeimbal (1939) found that young York Imperial apple trees with roots at 42° to 45°F. absorbed, assimilated, and translocated nitrogen after about four weeks. They suggested that Nightingale and Blake's trees might have done likewise if given sufficient time.

Brown (1939) found considerable differences in chemical composition and root and shoot growth of various species of grasses grown at low and high soil temperatures. Kentucky and Canada bluegrass made excellent root growth at a soil temperature of 50° or 60°F., while Bermuda grass made very little root growth. Root growth of Bermuda grass increased with each increase in temperature up to 100°F., but the bluegrasses were severely injured at this high temperature.

It is much easier to obtain evidence of species differences with respect to absorption than it is to explain why these differences exist. The writer has discussed the causes of decreased water absorption in a previous paper (Kramer, 1940). It was concluded that, while low soil temperature may hinder water absorption in several ways such as by reducing the water-supplying capacity of the soil, the rate of root extension, and the rate of active absorption, the principal cause of decreased water absorption at low temperatures probably is the increased resistance to water movement through the root cells. This increased resistance is caused in part by the increased viscosity of water as it is cooled and in part by decreased permeability of the root cells. It seems probable that there may be differences between species with respect to the changes in viscosity and per-

meability of the protoplasm caused by low temperature, just as there are species differences in respect to cold resistance. Levitt (1941) suggests that resistance to frost injury depends on the viscosity of the protoplasm being little increased and its permeability being little decreased by cooling. It may be that the protoplasm of cotton and watermelon undergo much greater changes in viscosity and permeability than the protoplasm of collards, thus resulting in a greater decrease in water absorption at low temperatures in cotton and watermelon than in collards.

The rate of cooling probably has considerable effect on the reaction to low temperature. Experiments with elm, privet and sunflower indicate that plants cooled to one or two degrees above freezing in four or five hours, or even over night, wilted severely, while plants cooled over a period of four or five days wilted but slightly. The rapidly cooled plants also transpired less than plants which were cooled slowly to the same temperature. Jones (1938) reported that gardenias which had been growing in warm soil wilted if suddenly cooled, but recovered their turgidity after a few days. It seems possible that slow cooling affords time for changes in protoplasmic properties to occur which tend to minimize the effects of cooling. This problem might be worth further investigation.

SUMMARY

An investigation was made of the effects of gradually cooling the soil on water absorption by collard, cotton, watermelon, sunflower, privet, elm, and red, white, loblolly and slash pine. Absorption was reduced in all species, but more in species which normally grow in warm soil than in species which normally grow at least part of the year in cold soil.

Watermelons and cotton absorbed only 20 per cent as much water at 10° as at 25°C., while Georgia collards absorbed 75 per cent as much at 10° as at 25°C. Loblolly and slash pine absorbed only 40 per cent as much water at 10° as at 25°C., while red and white pine absorbed 60 per cent as much at 10° as at 25°C.

The differences between species presumably result chiefly from differences with respect to the changes in viscosity and permeability of the protoplasm of the roots caused by low temperature.

Reduction in water absorption caused by cold soil is of considerable ecological and practical importance, because it often produces winter injury and even affects the growth of plants in greenhouses.

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PREHISTORIC MAIZE FROM CAÑON DEL MUERTO¹

Edgar Anderson and Frederick D. Blanchard

FROM ALMOST the beginning of archeological exploration in the southwest there have been reports of prehistoric maize. Through the kindness of Dr. Clark Wissler of the American Museum of Natural History we were able to examine one of the largest of these collections, made by Earl H. Morris in 1923 in Mummy Cave, Cañon del Muerto, Arizona. There are about two bushels of ears in the collection. Having been stored in a stone cist with a tight cover they are practically free from rodent and insect attack and are incredibly well preserved. Aside from their generally dull color (to be discussed below) they look as though they might have been grown only a few years ago. Actually they are very ancient. Though many details remain to be worked out it is now possible to give approximate dates to such prehistoric remains in the Southwest (Roberts, 1935). Morris is of the opinion (personal communication) that this large cache of maize "dates either from Basket Maker II or III. There were no associated objects to make a closer period designation possible. I see no line of reasoning to suggest that the cache was made after A.D. 700 while the position and character of the cist in which it was found would make a date of A.D. 500 or somewhat earlier more plausible."

At the American Museum the ears were heaped pretty much at random in a large glass display case, and, since not all of them could be examined in the time at our disposal, we divided them into two approximately equal portions. From one of these (222 ears) we measured every ear with the exception of some very small nubbins. Row number was scored for each ear. The gross length and width of each ear and the width of the kernel were determined with a celluloid rule and are only approximate. Especially is this true for length, since there were a number of broken ears whose original length could

not be determined. For assistance in scoring the ears and for much pertinent information we are indebted to Dr. Marcus M. Rhoades of Columbia University.

As in many collections of Basket Maker maize there was a slight tendency to fasciated ears, four being so markedly flattened that they were recorded as "flat ears" (table 1), and the records made on them have not been used in computing averages. Since there were only four of these in over 200 ears their inclusion, however, would scarcely have changed the averages.

Three main colors were present; there were 105 red, 114 brown, and 11 mosaic ears. Both the red and brown varied somewhat in color, and we sorted each into two classes, but, while the division between the reds and the browns is fairly objective, deciding just where the dividing line shall come between light and dark brown or light and dark red is pretty much a matter of personal opinion.

The brown ears are a curious dull chestnut brown (raw sienna of Ridgeway), somewhat the color of acorns, and are quite different from modern maize. The color lies in the endosperm and is a general characteristic of Basket Maker maize, having been discussed by Collins in his report (1921) on the prehistoric corn submitted to him by Guernsey and Kidder. Collins decided that this color represents either a previously unknown genotype or that it is due to "slow disintegration." Knowing that many modern Indians parch their maize before storing it (Cushing, 1920, p. 265), we attempted to produce similar colors in our own stocks of modern Indian varieties by parching the ears in a gas oven. With a Papago yellow flour corn we were able to match the exact shade of the light brown prehistoric ears as shown in figure 1. The resemblance shown in this photograph would be even more striking if the reproduction were in color. While some of the discoloration may be due to age, it nevertheless seems

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TABLE 1. Means and standard deviations for ear length, kernel width, and row number of maize from Cañon del Muerto, sorted according to color. It will be noted that the average differences are insignificant compared with the standard deviations.

	Number of flat ears	Number of specimens measured	Average ear length in cm.	Average width of kernel in mm.	Average number of rows
Deep red	1	50	10.9 ± 3.1	8.5 ± 1.0	12.2 ± 1.6
Red	0	51	12.2 ± 3.2	7.9 ± 0.9	12.2 ± 1.8
Mosaic	1	10	11.3 ± 2.4	8.7 ± 0.9	12.0 ± 1.8
Dark brown	1	41	11.8 ± 3.0	8.1 ± 0.7	12.9 ± 2.2
Light brown	1	67	11.1 ± 2.2	8.2 ± 1.0	12.0 ± 1.6
Total	4	222	11.4 ± 2.9	8.2 ± 1.0	12.3 ± 1.8

probable that its peculiarities of color are mainly due to its having been parched before it was stored. Part of the differences in color may be due to the severity of the parching; the rest is mainly caused by the differences in pericarp color. It is possible that the light brown originally had a white endo-

sperm, the dark browns a yellow endosperm. Both yellow and white endosperm are found today in the very similar maize grown by the Papago Indians.

According to Cushing, maize may be heated in three different ways before it is stored. It may be baked or steamed before it is completely mature (Cushing, loc. cit., p. 204-208) or it may be treated with dry heat after it is mature. "After the corn was ripened, two modes of making it eatable were extremely simple. Still on the ear it was toasted or in the kernel parched" (Cushing, loc. cit., p. 265). The maize from Cañon del Muerto had apparently been prepared in the second of these three ways. It does not have the translucent appearance of maize which has been steamed nor are the grains shrunken apart as when that process is employed. The grains have the dull appearance which can be matched by toasting mature maize with dry heat and some of the ears are slightly blistered along one side as if the heat had been too intense.

The variegated ears bore kernels similar to, if not identical with, those designated *mosaic pericarp* (P^{mo}) by Anderson and Ter Louw (1929). The red pericarp was apparently p^{rr} . In pericarp and endosperm color, the maize from this collection was practically identical with that discovered by Guernsey and Kidder and examined by Collins. While they did not report any mosaic ears the total number of ears in their collection was very small as compared with the more than 200 we have examined, and mosaic ears are known from other Basket Maker collections. In composition of the endosperm, however, the ears from Cañon del Muerto were quite different. All the material examined by Collins (1921, loc. cit.) was flinty, and, while we found a few flinty ears, they were very much in the minority. The change from flint to floury, however, though of great cultural importance, is genetically a relatively minor matter. As shown by Hayes and East (1915), it represents but a single gene difference, and it so greatly simplifies the preparation of corn meal that one would suppose it would immediately be selected in any variety of maize in which it arose, if climatic conditions permitted the cultivation of a flour corn. Because of the dominance relations between flinty and floury, the selection of a variety absolutely pure for floury would be a difficult matter, and it is not surprising that the flinty ears were encountered. In our

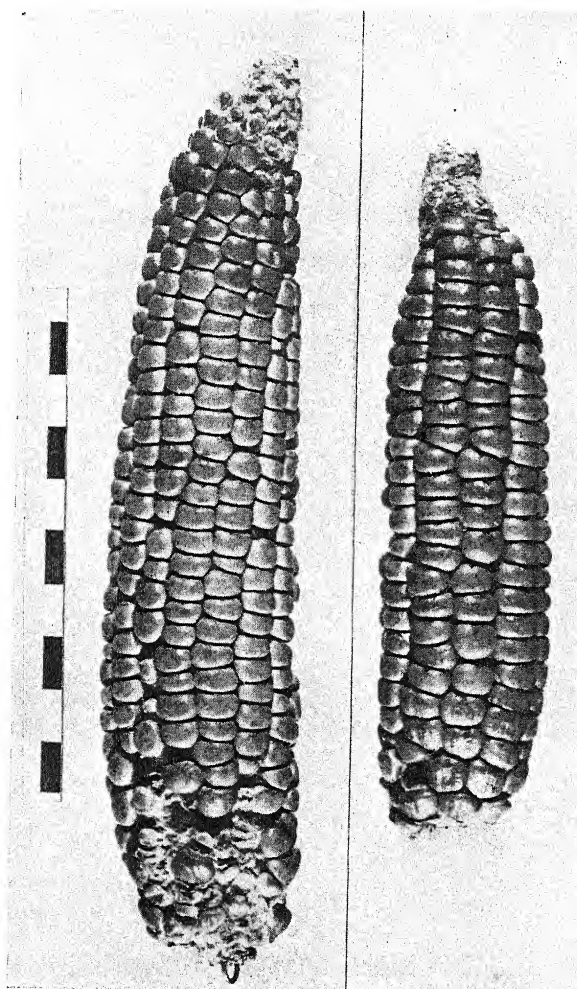


Fig. 1. Photographs of individual ears of modern Papago flour corn (larger ear with paper clip at base) and prehistoric maize from Cañon del Muerto. The Papago maize had been toasted in an oven.

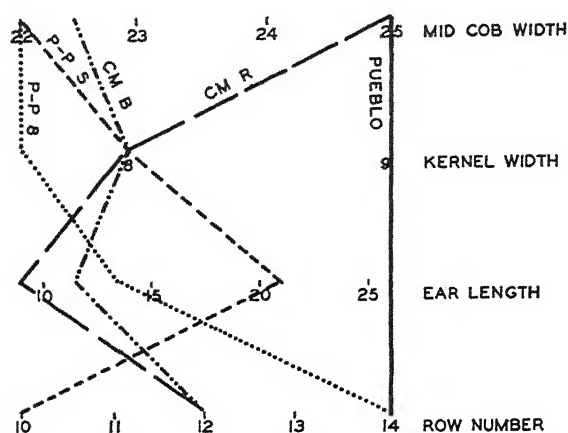


Fig. 2. Comparisons of prehistoric maize, modern Pima-Papago maize and modern Pueblo maize for four characters. Each line connects the average values for each character as follows: Pueblo. Unbroken line at right. Average values from Carter and Anderson collections of Pueblo maize.—Pima-Papago. (1) P-P 8—dotted line. Average values from Anderson's Papago inbred No. 8. (2) P-P S—short dashes. Average values from small-cobbed Pima-Papago varieties in Carter and Anderson collections.—Prehistoric. (1) CM R—long dashes. Averages of red ears from Cañon del Muerto. (2) CM B—dots and dashes. Averages of brown ears from Cañon del Muerto.

collections of the very similar flour corns grown by the modern Papago Indians, there are occasional ears with flinty kernels. The fact that the maize from Cañon del Muerto was prevailing flour corn suggests that it was culturally somewhat more recent than that described by Guernsey and Kidder (loc. cit.).

A single ear from the Cañon del Muerto collection has previously been identified as sweet corn by Erwin (1934). At the time of our visit it had been sent elsewhere and was not available for examina-

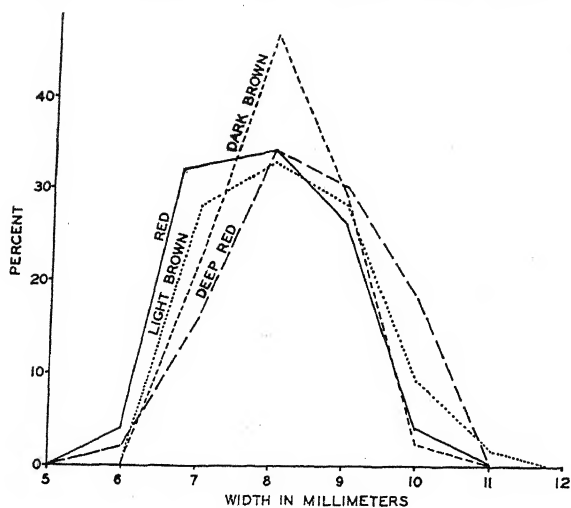


Fig. 3. Frequency distributions for kernel width in millimeters for the four color types.

tion. There were a few ears which at first sight looked as if they might be sweet corn, but closer examination showed them to be flint corn from which enough of the surface had cracked off to give it an irregular and translucent appearance not unlike that of sweet corn.

In its general appearance the maize from Cañon del Muerto is quite unlike that now being grown by the pueblo-dwelling Indians of the southwestern plateaus. Anderson and Cutler (1942) have recently discussed the races of maize in the southwest and have classified them into two main races, Pueblo and

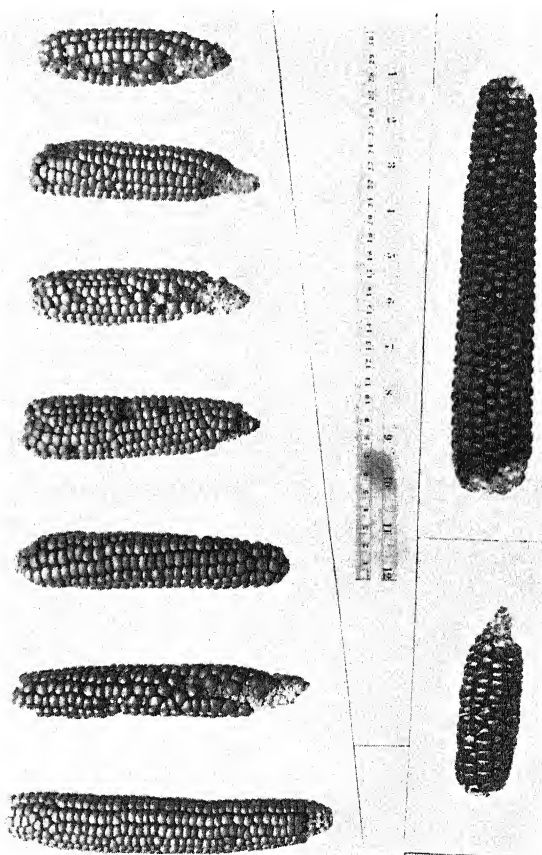


Fig. 4. Representative ears of prehistoric maize from Cañon del Muerto. (Photograph courtesy of the American Museum of Natural History.)

Pima-Papago. While maize from Cañon del Muerto may not be identical with that now grown by the Papago and allied tribes, it is certainly very similar. Exact comparisons for four measured characters are presented in figure 2, and figure 1 shows photographs of each. It will be seen that for the four measured characters the differences between the modern Papago and the prehistoric maize are not greater than those between the two colors of the prehistoric maize. All of the prehistoric maize, however, shows differences from that grown by the modern Pueblo Indians.

We made a special effort to see if there was any

evidence for a dual origin of the maize from Cañon del Muerto. If either the red or the brown had been the original type and the other color had been introduced with a second type, other differences would for some time tend to accompany the color differences, even though the two types were allowed to cross. In the maize of the modern Pueblos, for instance, there seem to be significant average differences in row number and cob size between the white varieties and the dark red varieties in our collections. If there is any such average difference in the material from Cañon del Muerto the figures do not bear it out, as is shown in table 1, and figure 3. If the color varieties represented in the maize from Cañon del Muerto had separate origins, their original differences in row number and cob size and shape were too slight or had been too completely merged by crossing to be apparent statistically.

SUMMARY

Two hundred twenty-two ears of prehistoric maize from Cañon del Muerto, Arizona, were examined in detail. Although nearly 1500 years old, the material is in an excellent state of preservation.

The dull colors of the prehistoric maize can be reproduced in modern maize by dry heat, and it is concluded that this cache had probably been parched before being stored.

Most of the maize had floury endosperm, though a few ears were flinty. Pericarp was either red, colorless, or mosaic (P^{rr} , P^{mo} , or p).

In cob size, kernel size, kernel shape, etc., this prehistoric maize is unlike modern Pueblo maize and very similar to that now being grown by the Papago and allied tribes.

A statistical analysis of differences within and between the color varieties of the prehistoric maize leads to the conclusion that, as a whole, it was essentially homogeneous.

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VITAMIN DEFICIENCIES OF CERATOSTOMELLA AND RELATED FUNGI¹

William J. Robbins and Roberta Ma

IN AN earlier paper (Robbins and Ma, 1942) we reported on the vitamin deficiencies of ten species of *Ceratostomella*. These species were found to suffer from partial or complete deficiencies for thiamine, for pyridoxine, for biotin or for two or more of these vitamins and were benefited by their addition to a basal medium. The present paper reports a survey of the vitamin deficiencies of thirteen additional species of *Ceratostomella* or related fungi.

MATERIAL AND METHODS.—The fungi investigated were received through the courtesy of the Division of Forest Pathology of the United States Department of Agriculture and were furnished by Dr. Ross W. Davidson who gave the following information on the cultures.

Ophiostoma catonianum G. Goid. This culture probably came from Goidanich.

Ceratostomella multiannulata Hedge. and David. Isolated from pine lumber in Louisiana.

Ceratostomella obscura David. Isolated from pine lumber in Louisiana.

Ceratostomella pilifera. Isolated from pine lumber in Louisiana.

Ceratostomella pluriannulata Hedge. Isolated from pine lumber in Louisiana in 1932.

Ceratostomella stenoceras Robak. This culture came from the Centraal Bureau voor Schimmel-cultures.

Ceratostomella microspora David.

Ceratostomella penicillata Grossman. Culture probably from Grossman in 1935 or 1936.

Grossmannia serpens G. Goid. Probably obtained from Goidanich.

Ceratostomella (Grossmannia) rostrocyndrica Davidson. Isolated from oak wood.

Endoconidiophora (Thielaviopsis) paradoxa.

Endoconidiophora coerulescens Munch. This culture originally came from the Centraal Bureau voor Schimmel-cultures.

Endoconidiophora adiposa (Butler) Davidson. Culture from sugar cane or water nut, series B-8 from Fred Andrus.

Dr. Davidson stated that none of these cultures was less than five or more than nine years old and some, for example *C. multiannulata*, had lost the ability to form perithecia.

¹ Received for publication August 24, 1942.

All cultures were tested for purity after being received, and those contaminated with bacteria were isolated in pure culture. No attempt, however, was made to prepare single spore isolations for the experiments reported in this paper. Stock cultures were maintained on a thiamine-peptone agar.²

² This is prepared by adding 625 m μ moles of thiamine and 1 g. of neopeptone per liter to the basal medium.

Each of these organisms was grown on agar slants in test tubes containing approximately 8 ml. of a basal medium, or the same medium to which thiamine, pyridoxine, biotin or all possible combinations of these vitamins were added. They were grown also on the basal medium plus Difco malt extract desiccated. In addition, those organisms which showed vitamin deficiencies were cultured in 25 ml. of the

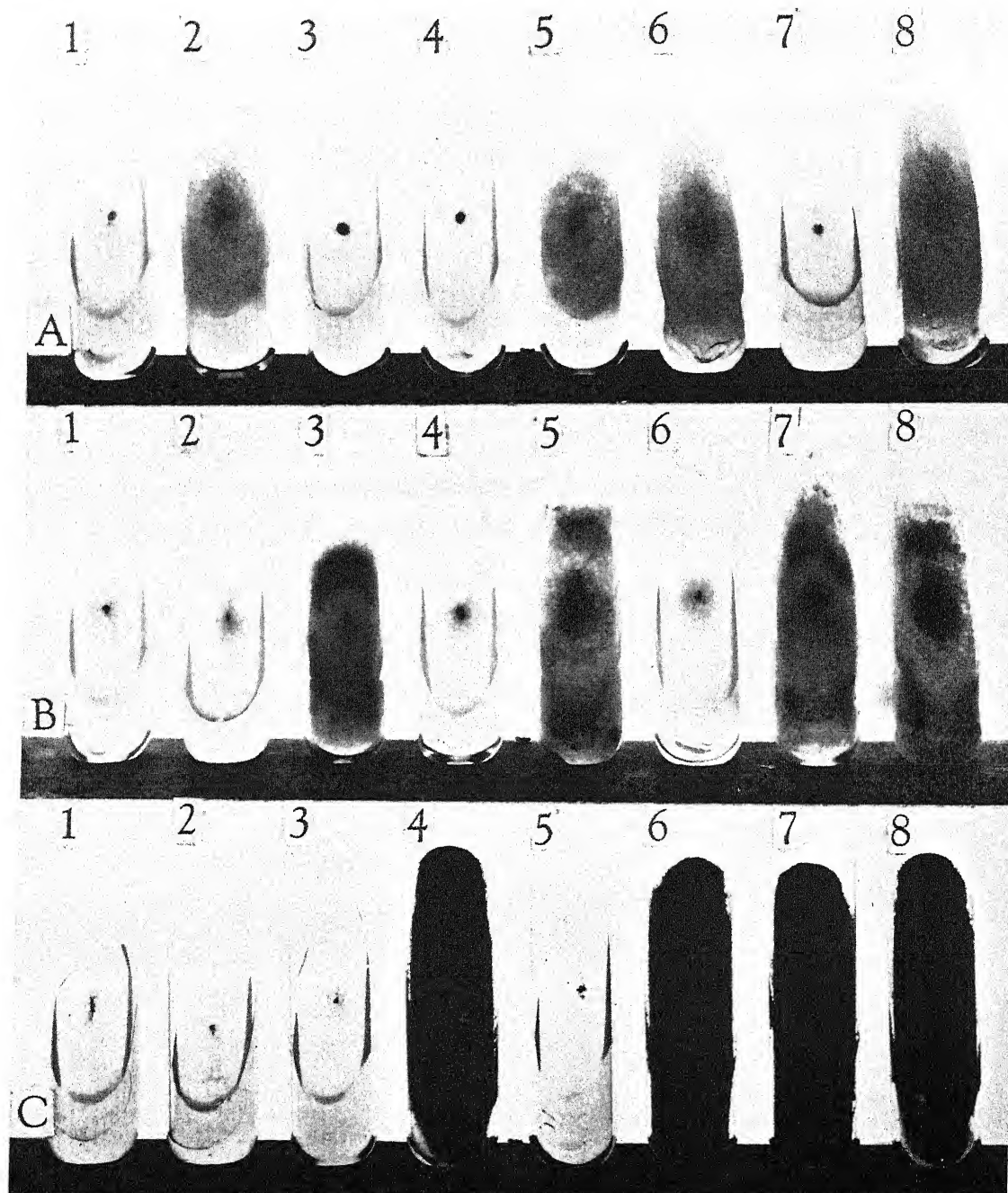


Fig. 1. Growth of three fungi on a mineral-dextrose medium containing asparagine and purified agar supplemented as follows: (1) no additions; (2) thiamine; (3) pyridoxine; (4) biotin; (5) thiamine and pyridoxine; (6) thiamine and biotin; (7) pyridoxine and biotin; (8) all three vitamins. A, *Cerastostomella stenoceras*; B, *C. pluriannulata*; C, *Grossmannia serpens*. All forty days old.

basal liquid medium in 125 ml. Erlenmeyer flasks to which the vitamins or malt were added. All cultures were in triplicate and incubated at 20°C. in the dark. All inoculations were made by transferring a bit of mycelium the size of a pin head except for the liquid cultures of *C. stenoceras*, *C. microspora* and *E. paradoxa* where a drop of a spore suspension in distilled water was used.

The basal medium contained per liter, 50 g. dextrose, 1.5 g. KH_2PO_4 , 0.5 g. MgSO_4 , 7H₂O and 2 g. asparagine. To this solution the following trace elements in p.p.m. were added: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn. For the agar cultures 1.5 per cent purified agar³ was added to the basal medium.

The dextrose was Corn Products C.P., the asparagine was purified by treatment with Norit A and recrystallization from redistilled alcohol. All other chemicals were of C.P. grade. The thiamine and pyridoxine were Merck's synthetic; the biotin methyl ester was a crystalline product from the S.M.A. corporation. Dry weights of the mycelium in the liquid cultures were obtained by filtering the mycelium into a Gooch crucible, washing with distilled water and drying at 100°C.

EXPERIMENTS.—The thirteen fungi were grown on agar slants in triplicate on nine different media as follows: (1) the basal medium, and the basal medium plus, per tube, (2) thiamine 5 m μ moles, (3) pyridoxine 5 m μ moles, (4) biotin methyl ester 0.05 μ g., (5) thiamine and pyridoxine, (6) thiamine and biotin, (7) pyridoxine and biotin, (8) thiamine, pyridoxine and biotin, (9) 0.3 g. malt extract. Observations were made at intervals on relative growth, and no cultures were discarded until no further development could be observed. All were observed at inter-

³ The purified agar was prepared by extracting Difco agar with 5 per cent aqueous pyridine followed by 1 per cent hydrochloric acid. The agar was then neutralized with calcium hydroxide and washed free of excess calcium.

vals over a period of forty days and some for a considerably longer time.

Eleven of the thirteen fungi showed marked deficiencies on the agar media for one or more of the three vitamins and these were grown in liquid cultures as follows: (1) in the basal medium and in the basal medium plus, per flask containing 25 ml. of medium, (2) 10 m μ moles of thiamine, (3) 50 m μ moles of pyridoxine, (4) 0.1 μ g. biotin methyl ester, (5) thiamine and pyridoxine, (6) thiamine and biotin, (7) pyridoxine and biotin, (8) thiamine, pyridoxine and biotin and (9) 0.3 g. malt extract. Observations were made of relative growth in the liquid cultures and dry weights of the mycelium were determined after from seven to forty-seven days incubation. The results were as follows:

Ceratostomella stenoceras appeared to be a thiamine-deficient organism. By the end of forty days (fig. 1) a small amount of subsurface growth had developed in the tubes containing the basal medium and that supplemented with pyridoxine, with biotin, and with pyridoxine and biotin. The surface of the agar slants was covered with mycelium in tubes of the basal medium supplemented with thiamine, with thiamine and pyridoxine, with thiamine and biotin, and with all three vitamins. Little difference was noted in the rapidity of growth on any of the media containing thiamine which suggests that partial deficiencies for pyridoxine and biotin do not exist for this fungus. The growth on malt agar was about like that on the agars containing thiamine. Growth in the liquid cultures (table 1) confirmed that on the agar media except for the liquid medium containing malt extract.

Grosmannia serpens on both the agar medium and in the liquid medium showed a complete biotin deficiency (fig. 1 and table 1). It grew rapidly and within eight days had covered the surface of the agar slants on those media containing biotin. Growth on

TABLE 1. Average dry weights of fungi grown at 20°C. in a mineral-dextrose solution containing asparagine supplemented as indicated in the table. *Ophiostoma* and *Endoconidiophora* grown seven days; *C. microspora* and *C. penicillata*, eighteen days; all others, twelve days.

Average dry weight per culture mg.										
Additions to 25 ml. basal solution	<i>Ceratostomella</i> <i>stenoceras</i>	<i>Ceratostomella</i> <i>pluriamulata</i>	<i>Ceratostomella</i> <i>multianulata</i>	<i>Grosmannia</i> <i>serpens</i>	<i>Ceratostomella</i> <i>obscura</i>	<i>Ceratostomella</i> <i>pilifera</i>	<i>Ophiostoma</i> <i>catonianum</i>	<i>Endoconidiophora</i> <i>paradoxa</i>	<i>Ceratostomella</i> <i>microspora</i>	<i>Ceratostomella</i> <i>penicillata</i>
None	3.6	0.3	0.1	2.2	2.5	2.1	0.1	3.3	0.1	0.3
10 m μ moles thiamine....	59.9	0.1	0.1	1.5	2.9	2.4	0.4	207.5	0.1	0.4
50 m μ moles pyridoxine...	2.0	0.8	0.1	0.1	0.3	0.5	52.3	1.4	0.1	0.2
0.1 μ g. biotin	1.2	0.9	2.5	91.5	3.2	37.5	0.3	0.9	0.1	0.3
Thiamine and pyridoxine..	45.3	67.7	18.4	2.2	1.0	2.1	48.4	195.2	1.3	0.6
Thiamine and biotin	46.9	0.4	0.5	110.3	26.1	100.7	0.4	197.9	0.1	3.4
Pyridoxine and biotin.....	0.7	1.8	0.5	114.5	3.2	124.6	57.0	0.6	1.4	0.5
Thiamine, pyridoxine and biotin	41.1	56.2	11.1	112.6	22.2	101.7	62.2	184.9	15.2	3.8
0.3 g. malt extract.....	11.7	119.6	61.0	133.8	48.4	47.2	38.3	213.8	25.9	48.5

malt agar or in the liquid medium containing malt was about the same as on the media containing vitamins.

Ceratostomella multiannulata appeared to suffer from a complete pyridoxine deficiency and a partial thiamine deficiency. The growth on the basal agar medium and on that supplemented with thiamine, with biotin, and with thiamine and biotin was scant

and colorless. More growth with a brownish color appeared on the pyridoxine medium and on that containing pyridoxine and biotin. Heavy growth covering the entire slant developed on the medium supplemented with thiamine and pyridoxine, and with all three vitamins (fig. 2). The growth on the malt agar was somewhat heavier than on the medium containing all three vitamins.

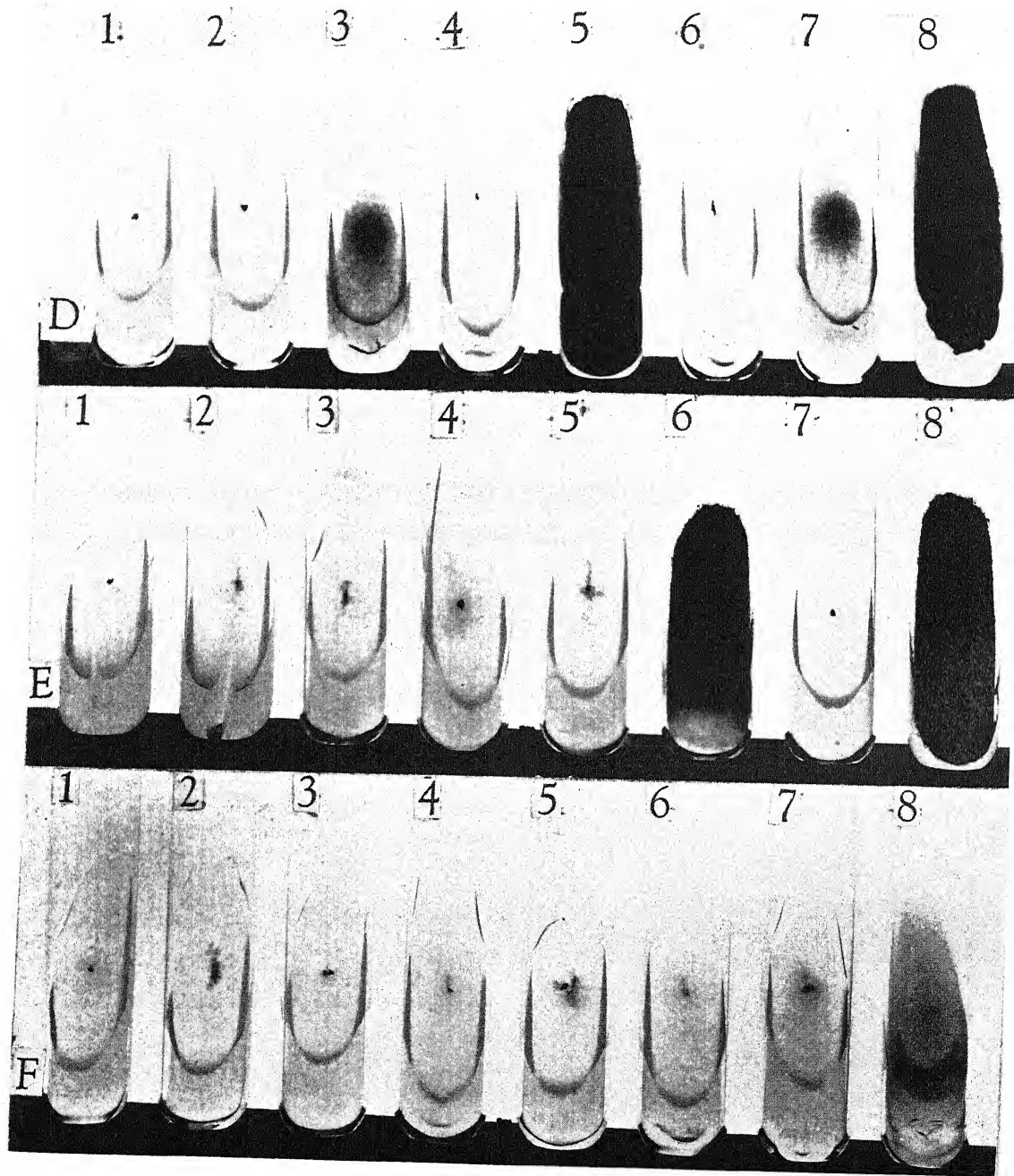


Fig. 2. Growth of three fungi on a mineral-dextrose medium containing asparagine and purified agar supplemented as follows: (1) no addition; (2) thiamine; (3) pyridoxine; (4) biotin; (5) thiamine and pyridoxine; (6) thiamine and biotin; (7) pyridoxine and biotin; (8) all three vitamins. D, *Ceratostomella multiannulata*; E, *C. penicillata*; F, *C. microspora*. All forty days old.

This organism grew slowly in liquid culture (table 1), and at the end of twelve days the maximum growth in the vitamin media was 18.4 mg. In the medium supplemented with malt it was 61.0 mg. which suggests that *C. multiannulata* has unidentified partial deficiencies. In general the growth in liquid culture was poorer than on agar. The dry weights taken at the end of twelve days (table 1) showed no benefit from the addition of pyridoxine, though this was evident on agar (fig. 2). Subcultures were made from the liquid cultures containing the basal medium and those supplemented with thiamine, with pyridoxine, and with biotin into similar solutions and allowed to grow forty-seven days. Although the growth was not great, the results (table 2) clearly showed benefit from pyridoxine.

TABLE 2. Average dry wts. of fungi grown forty-seven days at 20°C. in a mineral-dextrose solution supplemented as indicated.

Additions to 25 ml. basal solution	Ave. dry wt. per culture mg.	
	<i>Ceratostomella multiannulata</i>	<i>Ceratostomella pluriannulata</i>
None	0.1	0.2
10 m μ moles thiamine.....	0.1	0.0
50 m μ moles pyridoxine....	1.7	3.8
0.1 μ g. biotin	0.0	0.2

Ceratostomella pluriannulata resembled *C. multiannulata* in its relation to thiamine, pyridoxine and biotin, except that it grew somewhat more rapidly than the latter organism (fig. 1). Its response in liquid culture in contrast to that on agar suggests a complete deficiency for both thiamine and pyridoxine (table 1). However, subcultures made from the basal medium and from those supplemented with thiamine, with pyridoxine, and with biotin, which were allowed to grow forty-seven days, showed a small effect from the addition of pyridoxine (table 2). This fungus like *C. multiannulata* evidently synthesizes a little thiamine though not enough for maximum growth. It is possible that its ability to synthesize thiamine may be conditioned by the oxygen supply, that is by whether it grows in liquid or on agar. This is suggested because it appeared to develop more extensively on the agar medium supplemented with pyridoxine than in the liquid medium which contained the same vitamin. *C. pluriannulata* and *C. multiannulata* both showed a strong response to malt suggesting partial unidentified deficiencies.

After fifty-three days mature perithecia discharging ascospores were observed on the agar medium supplemented with thiamine and pyridoxine, with all three vitamins and with malt. None was observed on the media supplemented with pyridoxine, or with pyridoxine and biotin. Although the fungus grew fairly well (fig. 2) on the media containing pyridoxine, or both pyridoxine and biotin, it apparently needed the presence of both pyridoxine and thia-

mine to permit growth sufficiently vigorous for the formation of perithecia.

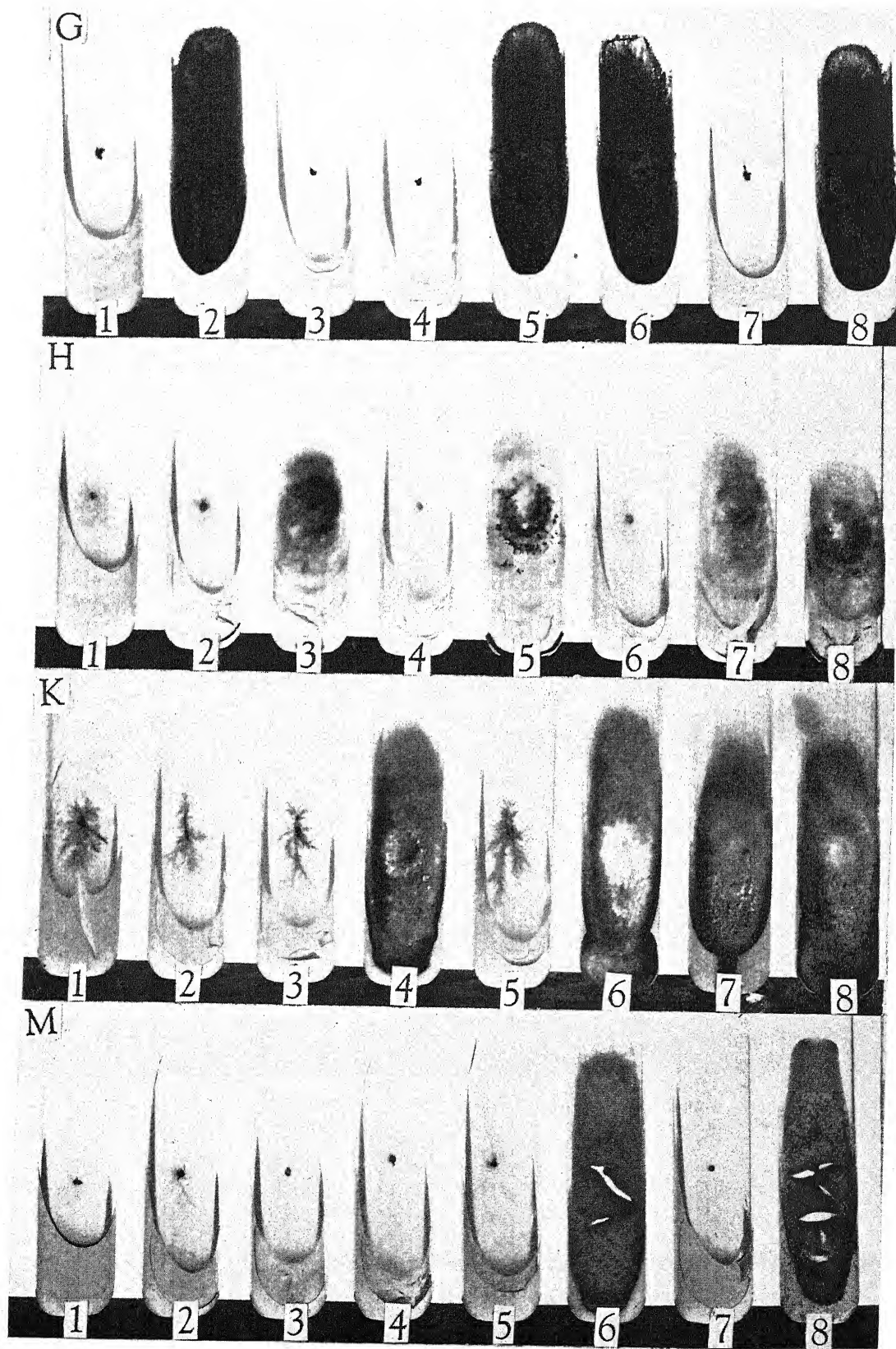
Ceratostomella penicillata formed little mycelium on the basal agar medium and on that supplemented with thiamine, with pyridoxine or with thiamine and pyridoxine. The growth with biotin and with pyridoxine was somewhat better but still very scant. Growth on the medium with thiamine and biotin was good (fig. 2). Somewhat more rapid development was observed on the medium with all three vitamins and still better on the malt agar. On the agar media *C. penicillata* suffered from a complete or nearly complete deficiency for thiamine and biotin. This was confirmed by the growth in liquid cultures, though the dry matter produced even in eighteen days was small. It responded markedly to the malt extract in both the agar and liquid medium.

Ceratostomella microspora grew little on the basal agar medium or on that supplemented with thiamine, with pyridoxine, or with pyridoxine and thiamine. Slightly greater growth was observed on the medium to which biotin, or both thiamine and biotin were added and somewhat better growth with a little surface mycelium developed on the agar medium supplemented with pyridoxine and biotin. However, not until all three vitamins were added did much mycelium develop (fig. 2) and the growth on malt agar was considerably better. This fungus appeared to suffer from complete or nearly complete deficiencies for thiamine, pyridoxine and biotin and in addition from partial deficiencies for unidentified factors in malt. These conclusions were confirmed by the liquid cultures (table 1).

After eighty-seven days mature perithecia in considerable numbers were observed on the agar medium supplemented with all three vitamins. They were produced in a circle about 1.5 cm. in diameter with the point of inoculum as the center.

Endoconidiophora paradoxa grew very rapidly on the agar media containing thiamine. At the end of four days very little growth had appeared in the tubes containing the basal medium, or that supplemented with pyridoxine, with biotin, or with biotin and pyridoxine. In all others the surface of the slant was covered with a heavy black mycelium. This picture remained unchanged for more than thirty days during which the tubes were under observation (fig. 3).

The same results were obtained in liquid culture (table 1). About 200 mg. of mycelium (dry weight) were obtained in the cultures which contained thiamine, or thiamine and one or both of the other vitamins, and also in the malt medium. In the solutions lacking thiamine the maximum dry weight was 3.3 mg. *E. paradoxa* appeared to be a thiamine-deficient organism. Its growth on media containing malt was slightly better than on the media containing vitamins. Its rapid growth, large dry weight and general character suggests that it might be investigated further as a means of bio-assay for thiamine or its intermediates.



Ophiostoma catonianum on the agar medium appeared to suffer from a complete pyridoxine deficiency and a partial deficiency for thiamine. Its development during the first week or ten days was somewhat better on the medium containing pyridoxine and thiamine or all three vitamins than on that supplemented with pyridoxine alone. It grew a little better on malt agar than on agar containing the three vitamins. At the end of twenty-eight days the growth on the basal medium and on that supplemented with thiamine, with biotin, or with thiamine and biotin, was thin and waxy, 12 or 15 mm. in diameter. On all the media containing pyridoxine the growth was thick and cottony. The graphium stage was freely produced on the medium supplemented with thiamine and pyridoxine, with pyridoxine and biotin, and with all three vitamins, but formed scantily on the media containing pyridoxine or malt. The appearance of *O. catonianum* on the various agar media is shown in figure 3 at the end of eighteen days' incubation. Its appearance was essentially unchanged after forty days' incubation.

These results were substantiated in general by the growth in liquid culture (table 1). The cause for the poorer growth in the liquid medium containing malt as compared to that in the medium supplemented with all three vitamins was not investigated. The dry weights show the complete pyridoxine deficiency but do not furnish evidence for the partial thiamine deficiency observed on the agar cultures.

Ceratostomella pilifera showed on the agar media a complete biotin deficiency and perhaps partial deficiencies for thiamine and pyridoxine. During the first week or ten days little growth developed on the basal medium or on that supplemented with thiamine, with pyridoxine, or with thiamine and pyridoxine. The growth on the medium containing biotin was good; it was a little better on that supplemented with biotin and thiamine, with biotin and pyridoxine, and with all three vitamins. The growth on the malt agar was a little heavier than on any of the media supplemented with vitamins. At the end of twenty-eight days a scant growth of black rhizoid-like mycelium had developed in the basal agar medium and in that supplemented with thiamine, with pyridoxine, or with thiamine and pyridoxine. On the media supplemented with biotin, with biotin and thiamine, with biotin and pyridoxine, with all three vitamins, and with malt, the agar slants were covered with a thick gray cottony mycelium with a powdery surface growth (fig. 3).

In the liquid cultures little growth was obtained without the addition of biotin. However, a considerably greater (about three times greater) growth developed when thiamine or pyridoxine or both were present with the biotin. This suggested partial deficiencies for thiamine and for pyridoxine, the satisfaction of either of which resulted in a greater syn-

thesis of the other. We do not believe that this may be taken as evidence that pyridoxine replaces thiamine or vice versa. In contrast to the results on agar less growth was obtained in the liquid cultures containing malt than in those supplemented with vitamins. A different amount of malt might have given better results.

Ceratostomella obscura evidenced a complete deficiency for thiamine and biotin. By the end of twenty-eight days a delicate widely spreading mycelium had developed in the tubes containing the basal medium and those supplemented with thiamine, with pyridoxine, with biotin, with thiamine and pyridoxine, or with pyridoxine and biotin. This slight growth may have developed from the vitamin supplied with the inoculum. The surface of the agar slants on the tubes containing thiamine and biotin, and on those containing all three vitamins, was covered with a yellowish-brown waxy growth with little aerial mycelium. In the tubes containing malt extract the medium was covered with growth and considerable aerial mycelium had developed (fig. 3). The malt agar appeared to be somewhat superior to the vitamin medium. The growth in liquid culture (table 1) confirmed the observations made on the agar cultures. Growth in the liquid medium containing malt was markedly superior to that in the media containing vitamins.

Endoconidiophora adiposa grew rapidly on the basal agar medium. Within four days the agar slants were covered with a gray fluffy mycelium. Little effect of the addition of any one or all of the vitamins or of malt extract was noted.

Endoconidiophora coerulescens grew on the basal agar medium. Its development on the malt agar was more rapid. It appeared to show some response to thiamine but the thiamine deficiency was slight.

Ceratostomella (*Grosmannia*) *rostracylindrica* grew slowly on the basal agar medium. After two weeks the colonies were only 8 mm. in diameter and after thirty-four days they had increased to 15 mm. None of the vitamin additions had any effect, but the growth on malt agar was about twice that on the basal medium.

DISCUSSION.—Eleven of the thirteen fungi used in this investigation showed partial or complete deficiencies for one or more of the three vitamins, thiamine, pyridoxine and biotin (table 3). Four showed a complete or nearly complete deficiency for thiamine, six for pyridoxine, and four for biotin. In addition, partial deficiencies for thiamine were evidenced by five, and for pyridoxine by one. Two of the fungi had complete deficiencies for two of the vitamins and one for all three. In this investigation and the one reported earlier, species of *Ceratostomella* and related organisms have been found which evidence nearly all possible combinations of partial and complete deficiencies for thiamine, pyridoxine

Fig. 3. Growth of four fungi on a mineral-dextrose medium containing asparagine and purified agar supplemented as follows: (1) no addition; (2) thiamine; (3) pyridoxine; (4) biotin; (5) thiamine and pyridoxine; (6) thiamine and biotin; (7) pyridoxine and biotin; (8) all three vitamins. G, *Endoconidiophora paradoxa*; H, *Ophiostoma catonianum*, K, *Ceratostomella pilifera*; M, *C. obscura*. *Endoconidiophora paradoxa* six days old; others eighteen days old.

TABLE 3. Vitamin deficiencies of eight species of *Ceratostomella* and five related fungi and the effect of malt extract.

Fungus	Thiamine	Deficiency for Pyridoxine	Biotin	Effect of malt extract
<i>Ophiostoma catenium</i>	Partial	Complete	None	None
<i>C. obscura</i>	Complete	None	Complete	Marked in liquid media
<i>C. pilifera</i>	Partial?	Complete	None	Positive on agar
<i>C. multiannulata</i>	Partial	Complete	None	Marked
<i>C. stenoceras</i>	Complete	None	None	Slight or none
<i>C. pluriannulata</i>	Partial	Complete	None	Marked
<i>C. penicillata</i>	Nearly complete	Partial?	Complete	Marked
<i>C. microspora</i>	Nearly complete	Nearly complete	Complete	Considerable in liquid medium
<i>C. rostricylindrica</i>	None	None	None	Great
<i>Grosmannia serpens</i>	None	None	Complete	Slight or none
<i>Endoconidiophora paradoxa</i>	Complete	None	None	Some
<i>Endoconidiophora coerulescens</i>	Partial?	None	None	Marked
<i>Endoconidiophora adiposa</i>	None	None	None	None

and biotin. The results emphasize the importance of these three vitamins as constituents of the medium for this group of fungi and explain why media containing natural supplements have been so freely and successfully used in their cultivation.

Little previous work on the vitamin deficiencies of these organisms has been reported. Hawker found that the addition of a lentil extract to a mineral-dextrose medium containing agar increased the production of pycnidia by *Ceratostomella adiposum*. The inositol-free extract was ineffective (Hawker, 1936).

Leonian and Lilly (1938) were unable to grow *Ceratostomella multiannulata* on a mineral-dextrose medium containing NH_4NO_3 and agar or on the same medium supplemented with thiamine, riboflavin, an amino-acid mixture or thiamine with amino-acids. Growth occurred when yeast extract was added to the agar medium, and they tentatively listed this fungus as a biotin-deficient organism (1940). Our results do not indicate a biotin deficiency for this fungus but a complete pyridoxine deficiency and partial thiamine deficiency.

In spite of the primary importance of the three vitamins mentioned for most of the fungi included in this study, it should be noted that some of them grew better on a malt agar than on the agars supplemented with vitamins. This suggests that these organisms may suffer from partial deficiencies for unidentified growth substances present in the malt, a possibility which was not investigated.

Attention was called to certain differences between the effect of the vitamins in the agar medium and in the liquid medium. These differences were quantitative rather than qualitative and may indicate that the power of some of the fungi to synthesize a particular vitamin from elementary substances is conditioned by the oxygen supply. A conditioned deficiency of this type has been described for *Staphylococcus aureus* in its relation to uracil (Richardson, 1936). On the other hand, the difference

may not be as great as appeared. Growth on agar slants can be estimated but not measured accurately in terms of dry weight; a small amount of mycelium spread over the surface of an agar slant may give the impression of a greater dry weight than actually exists.

C. pluriannulata produced perithecia when furnished thiamine and pyridoxine; it grew quite well but formed no perithecia on an agar medium supplemented with pyridoxine. Hawker (1939) found that *Melanospora destruens* grew on a medium supplemented with biotin but formed no perithecia; perithecia were produced on a medium containing biotin and thiamine. These observations show a relation between vitamin supply and the production of sex organs by certain fungi. It is doubtful, however, that this relation is direct. We are inclined to regard failure to produce perithecia in the absence of thiamine, for example, as a symptom of a fundamental disturbance in the physiology of the organism. We anticipate that other vitamins may be found associated with the production of sex organs by other fungi, and that the vitamins which are found to be active will depend upon the deficiencies of the organism concerned.

We may assume on the basis of previous results (Robbins and Ma, 1942) that each fungus is able to synthesize from the constituents of the basal medium those vitamins for which it shows no deficiency, and some, but inadequate, amounts of those for which it evidences a partial deficiency. *Ceratostomella stenoceras* and *Endoconidiophora paradoxa*, for example, without doubt synthesize biotin and pyridoxine when grown in a thiamine medium. *Ophiostoma catenium* may be assumed to synthesize thiamine and biotin when grown in a medium containing pyridoxine.

It appears clear that in a mineral-dextrose medium containing asparagine the growth of the majority of these fungi is limited by the supply of thiamine, pyridoxine or biotin. However, the question as to what limits their growth when these three

vitamins are available is unanswered. Why, for example, is the growth of *Ceratostomella penicillata* so much slower in the vitamin medium (or the malt medium) than that of *Endoconidiophora paradoxa*? Our observations raise many problems which require a detailed investigation of individual organisms.

SUMMARY

Thirteen species of *Ceratostomella* and related fungi were grown in a mineral-dextrose medium containing asparagine, and in the same medium supplemented with thiamine, pyridoxine or biotin, singly and in combination, or with malt extract. Eleven of the species suffered from complete or par-

tial deficiencies for one or more of the three vitamins. Six had complete or nearly complete deficiencies for pyridoxine, four for thiamine, and four for biotin.

Ceratostomella obscura showed a complete deficiency for pyridoxine and biotin, and *C. microspora* for all three vitamins. Six of the fungi grew markedly better in a medium supplemented with malt than in media supplemented with the three vitamins.

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OBSERVATIONS ON THE FLOWERING HABITS OF FOUR PASPALUM SPECIES ¹

Glenn W. Burton

A STUDY of the blooming process in plants often results in the discovery of features peculiar to individual species which may be utilized to facilitate the manipulation and hybridization of those species in a breeding program. Since May, 1936, when the grass breeding project at the Georgia Coastal Plain Experiment Station, Tifton, Georgia, was initiated, numerous observations on the anthesis of the improved grasses in the Southeast have been recorded. In this paper the blooming behavior of Dallis grass, *Paspalum dilatatum* Poir. Vasey grass, *Paspalum urvillei* Steud.; Bahia grass, *Paspalum notatum* Flügge; and *Paspalum malacophyllum* Trin. as related to certain climatological factors is presented.

Many publications dealing with the flowering habits of the cereals and more common northern grasses appear in the literature. In the absence of papers describing the flowering habits of the Paspalums, some of these publications have been reviewed and will be considered in the interpretation of the data presented in this paper.

OBSERVATIONS.—With the exception of a few potted plants used in dark-room studies, all of the follow-

¹ Received for publication August 29, 1942.

Cooperative investigation at Tifton, Georgia, of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the Georgia Coastal Plain Experiment Station, and the Georgia Experiment Station. The writer is indebted to Fred Bell, Jr., Agent, U. S. Dept. of Agriculture, Tifton, Georgia, for assistance in making many of the observations reported herein.

ing observations were made on well established field plantings of each of the species growing at Tifton, Georgia. Night observations were made by subjecting the panicles to light from a small flashlight at intervals only long enough to procure the necessary records. Representative panicles of the four *Paspalum* species considered here are shown in figure 1.

Heading period.—The cereals and many of the northern cultivated grasses head at a definite season of the year and complete their blooming process in a relatively short period of time (Evans, 1916; and Evans and Ely, 1941), usually from one to three weeks. The *Paspalum* species considered here, with the exception of the Paraguay strain of Bahia grass, begin to head in May and June and, except under very adverse environmental conditions, produce heads continuously until late October. The strain of *Paspalum notatum* from Paraguay has a major heading period of about four weeks followed by the formation of few if any panicles.

Table 1 indicates that the initial heading date in these Paspalums may vary a month or more from year to year depending upon certain climatological factors. Since all of these grasses head on both longer and shorter days and at higher and lower temperatures than those experienced in May and June, it is evident that photoperiod or temperature level are not directly responsible for the initiation of panicles in these grasses each spring.

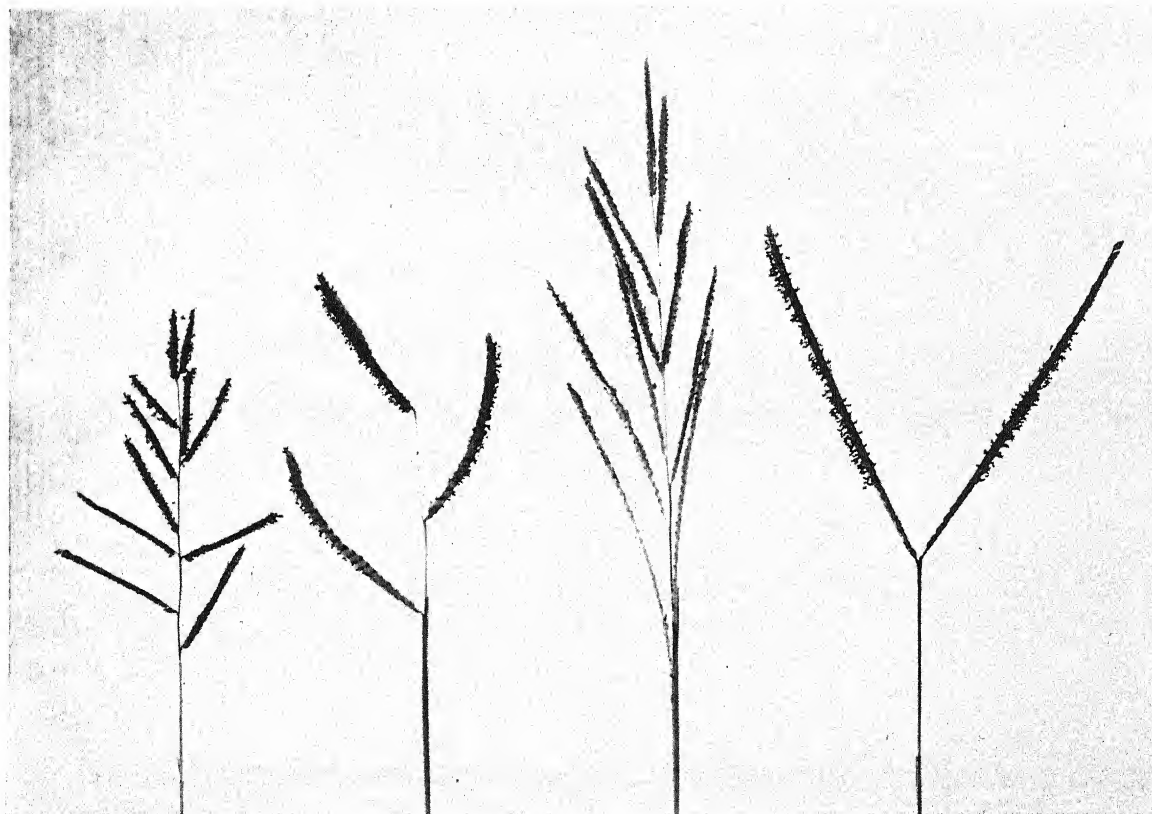


Fig. 1. Left to right are representative panicles of *Paspalum malacophyllum*, *P. dilatatum*, *P. urvillei*, and *P. notatum*, $\times \frac{1}{2}$.

Evans (1939) working with timothy has shown that "for each degree of average daily temperature above the mean (during April, May and June), the average date of full bloom was advanced two days earlier than the medium, and for each degree below the mean, the date when the maximum number of plants were in full bloom was delayed two days. A somewhat similar relation between spring temperatures and heading date was observed in these Paspalum

lums in 1938 and 1939, but the severe winter injury in 1940 and the April-May drought of 1941 apparently obscured these spring temperature effects. These observations suggest that any climatological factor that noticeably delays spring growth such as winter injury, low spring temperatures, and spring drought will delay heading in these Paspalums.

Blooming process.—Under favorable conditions for blooming all of the Paspalums in this study fol-

TABLE 1. The influence of certain climatological factors upon the initial heading dates for several *Paspalum* species at Tifton, Georgia, from 1938–1941.

Observation period	Climatological factors			Date heads first appeared on				
	Winter temperatures	Mean spring temperature ^a	Total Apr.-May rainfall	<i>P. dilatatum</i>	<i>P. urvillei</i>	<i>P. malacophyllum</i>	<i>P. notatum</i>	<i>P. notatum</i>
		F.	inches				(Paraguay) ^c	(common)
1938	Moderate	69.0	4.72	May 1	May 5	May 21	May 21	June 1
1939	Mild	65.4	9.98	May 5	May 10	May 28	June 1	June 7
1940	Severe ^b	77.0	5.40	May 15	May 18	June 7	June 18	June 25
1941	Mild	64.1	2.70	May 18	May 21	June 11	June 10	June 20
Average 1938–1941				May 10	May 13	June 1	June 5	June 13

^a March 1 to May 31.

^b All of the grasses listed here were injured sufficiently to retard their spring growth.

^c The Paraguay strain of *P. notatum* has a major heading period of about four weeks followed by the formation of few if any seed heads. The other Paspalums listed above usually continue to produce heads until October each year.

low the same general blooming procedure. To facilitate the recording of observations this procedure has been divided into four empirical stages which are demonstrated with florets of *P. notatum* in figure 2. The first stage in this process consists of the

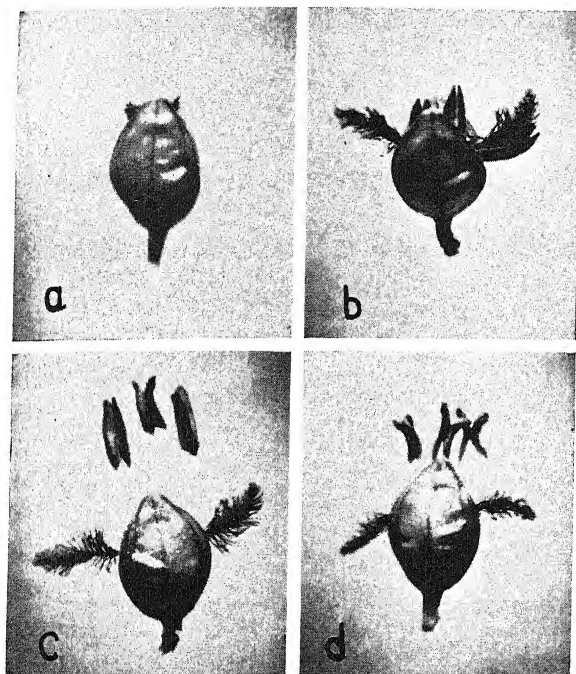


Fig. 2. Florets of *Paspalum notatum*, $\times 6$, demonstrating empirical stages in the blooming process of the Paspalums.—a. At stage 1 the glumes begin to open.—b. At stage 2 the filaments begin to elongate exerting the anthers.—c. At stage 3 the anthers are completely exerted.—d. At stage 4 the anthers dehisce shedding pollen.

opening of the fertile floret. The beginning of the exertion of the anthers marks stage 2. At stage 3 the anthers are completely exerted, and in stage 4 the anthers dehisce shedding pollen. Usually the glumes close soon after the pollen is shed.

Under less favorable conditions many variations from the normal blooming process have been observed. In a dry atmosphere the anthers usually dehisce very soon after the glumes open and before they are completely exerted. Frequently, under such circumstances, the glumes will close so quickly after opening that none or only small portions of the anthers and stigmas will be exerted. Flowers which have retained one or more of the anthers or stigmas within the glumes are frequently found in these grasses, particularly in *P. notatum*.

Order of blooming.—The order of blooming in these Paspalums has been observed many times. Usually less than half of the florets on any one raceme bloomed in any twenty-four-hour period, and frequently three to four days elapsed between the initiation and completion of anthesis in any one raceme. There was a marked tendency at all times for the florets in the terminal portion of the raceme to

bloom first, but frequently the florets at the very tip of the raceme did not bloom until the second or third day.

In *P. malacophyllum*, *P. dilatatum* and *P. urvillei* which have more than two racemes per panicle (fig. 1), anthesis began in several of the uppermost racemes. These three species also have paired spikelets crowded into four rows along the entire length of the raceme. Here the outer spikelet of the pair almost invariably bloomed one or more days earlier than the inner spikelet.

The average number of days required to complete anthesis in single panicles of these *Paspalum* species in June, 1941 (based on observations on 25 panicles of each grass), was 4.3 for common *P. notatum*, 4.6 for Paraguay *P. notatum*, 5.6 for *P. dilatatum*, 8.1 *P. malacophyllum* and 8.2 for *P. urvillei*.

On the basis of extensive studies with a number of northern cultivated grasses (*Phleum*, *Poa*, *Festuca* and *Lolium* species) Fruwirth (1916) concluded that blooming begins in the upper third of the inflorescence, at the end of a single branch in panicles and is completed in any one panicle in from four to eight days, depending upon the species. The similarity between these northern and southern grasses is apparent.

Time of blooming.—The hour at which each floret on twenty panicles of *P. notatum* bloomed was recorded from 4 A.M. to 8 P.M. for the period July 27 to August 1, 1936. To facilitate record taking anthers were removed from each flower as soon as its blooming was recorded. Weather records taken at hourly intervals for this period included air temperature, soil temperature at a depth of two inches, relative humidity and general weather conditions, whether clear, cloudy, or rainy. These data are presented graphically in figure 3.

From July 27 to July 30, a period of fair weather, 97.6 per cent of the 1190 flowers that bloomed, did so between 6:00 and 8:00 A.M. On August 1, a cloudy day with slow intermittent showers, the blooming was spread over a much longer period of time and only 31.2 per cent of the 144 flowers blooming on that day bloomed between 6:00 and 8:00 A.M. Although, as figure 3 indicates, a few florets bloomed on the afternoons and nights of the rainy days, most of the *P. notatum* flowers bloomed between 6:00 A.M. and 12:00 noon. Many more recent observations indicate that the above statements generally describe the blooming behavior of this species.

General observations made on plantings of *P. dilatatum*, *P. urvillei* and *P. malacophyllum* from July 27 to August 1, 1936, revealed that practically all of the florets of these grasses blooming in any twenty-four-hour period bloomed early in the morning. *Paspalum malacophyllum* was the only grass that failed to bloom on the rainy days of July 31 and August 1.

From June 15 to 24, 1939, the anthesis of the Paspalums considered here was carefully observed from 3:30 to 8:30 A.M. The hour when the majority of the florets started to bloom and the time in minutes

TABLE 2. The average hour, Eastern Standard Time, when anthesis began and the average time in minutes required to reach blooming stages 2, 3 and 4 (see figure 2) in several *Paspalum* species at Tifton, Georgia, during the ten-day period July 15-24, 1939.^a

Stage of anthesis (see figure 2)	Hour of initial blooming and minutes required to reach subsequent stages in:				
	<i>Paspalum malacophyllum</i>	<i>Paspalum dilatatum</i>	<i>Paspalum urvillei</i>	<i>Paspalum notatum</i>	<i>Paspalum notatum</i>
	A.M.	A.M.	A.M.	(common) A.M.	(Paraguay) A.M.
1	3:58 ^b	6:25	5:12	6:19	5:32
2	5.4	5.7	5.8	7.0	7.7
3	13.2	12.0	10.9	13.0	15.4
4	19.6	18.9	16.7	21.4	26.1

^a The records above apply to the period when the majority of the florets were blooming. Usually a number of florets bloom both before and after the period of intense blooming, particularly in *P. urvillei* and *P. malacophyllum*.

The sun arose about 5:30 A.M. Eastern Standard Time during the course of this study.

^b On two days following rains of the previous night *P. malacophyllum* did not bloom and on two other days it had finished blooming by 3:30 A.M., the time when the first observations were taken.

required to reach subsequent blooming stages were recorded for each grass for each day of the observation period. The ten-day average of these data is presented in table 2. During this ten-day observation period rather large day to day variations in the hour when blooming began and in the time required to reach different stages of blooming were observed.

The differences between the earliest and latest hour when blooming began exceeded one hour for *P. malacophyllum*, *P. urvillei* and *P. dilatatum* and amounted to twenty-six and fifty-three minutes for the common and Paraguay strains of *P. notatum*. Anthesis was observed to progress at a faster rate on warm mornings than on cool cloudy mornings.

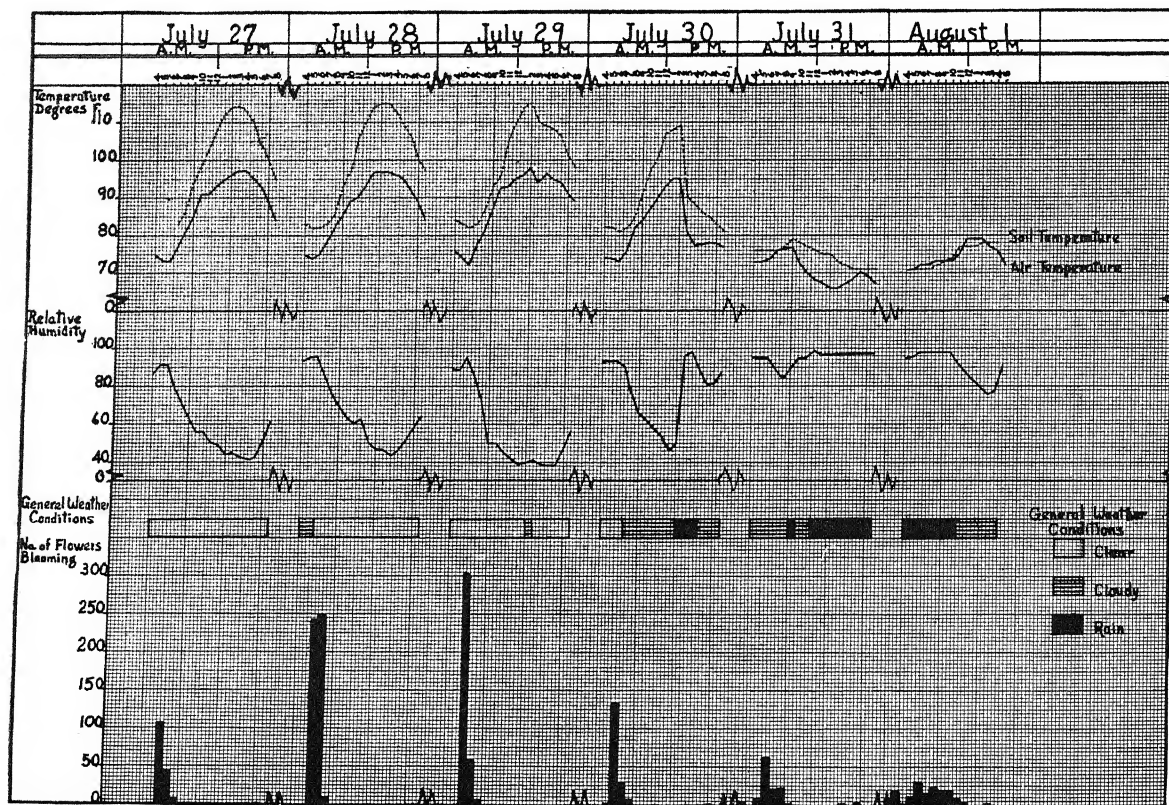


Fig. 3. The hours at which the florets on twenty panicles of *Paspalum notatum* bloomed in the field at Tifton, Georgia, for the period July 27 to August 1, 1936, as related to certain features of the environment.

According to Beddows (1931), Fruwirth (1916), Jenkin (1924) and Wolfe (1925) many of the northern cultivated grasses bloom most abundantly in the early morning. Fruwirth (1916), working in Germany, observed that most of these species had a secondary, less intense blooming period in the afternoon, usually from 4:00 to 6:00 P.M. He reported that the blooming of a single floret usually lasted one to two hours and concluded that weather conditions exercised "a great influence" on the time of blooming. The *Paspalums* considered here bloomed in the early morning, did not have a secondary blooming period in the afternoon and completed anthesis in single florets within fifteen to thirty minutes. Weather conditions, as in the case of the northern grasses, influenced the time and rate of blooming.

ADDITIONAL OBSERVATIONS.—Potted plants of *P. dilatatum*, *P. urvillei*, and both strains of *P. notatum*, when placed in a photographic darkroom in the late afternoon, bloomed little if any on the following day unless exposed to light. When subjected to daylight before 8:30 A.M. on clear days (as late as noon on cloudy days) or to a 100 watt Mazda light in the darkroom, these panicles bloomed normally. In each instance air temperatures were not above 80 degrees Fahrenheit. Later in the day when air temperatures were above 80 degrees F. those florets exposed to light either failed to bloom or opened and closed so rapidly that the floral organs were never completely exerted.

In the field, light-proof paper bags placed on panicles of these *Paspalums* in the late afternoon delayed flowering on the following morning. If on the following day these bags were removed between sunrise and 8:30 A.M. on clear days or before noon on cloudy days, normal blooming usually followed. When these bags were removed later in the day at air temperatures above 80 degrees Fahrenheit, the panicles either failed to bloom or bloomed abnormally.

Some florets of *P. urvillei* usually bloomed in the darkroom or in light-proof bags. This response was expected, since many florets on unbagged panicles of this species frequently bloom before dawn.

Paspalum malacophyllum has invariably failed to bloom on rainy days or on days immediately following rainy days, whether in the field or in the greenhouse. The other four *Paspalums* have bloomed in the greenhouse during heavy rains and have been observed blooming between showers in the field.

On foggy or misty mornings the florets of these grasses have remained in stage 3 of the blooming process (the anthers exerted but not dehiscent) much longer than usual. In order to determine experimentally the effect of mist upon anthesis, panicles of *P. notatum* and *P. dilatatum* were placed in a large cloth-covered box in which an atomizer created a continuous fine mist of water. On clear days when untreated panicles had bloomed by 8:00 A.M., florets on panicles enclosed in this artificial mist box started to bloom but remained in stage 3 until they

were removed from the mist at noon. These observations suggest that stage 4 in the blooming process (the dehiscence of the anthers) depends upon desiccation and may be greatly delayed in a mist-laden atmosphere.

Since these *Paspalums* bloom and shed pollen in the early morning, it is often difficult to collect pollen for hybridization purposes without getting it wet with the dew accumulated on the plants. When panicles of these grasses were cut, placed in water and brought into the laboratory either during or a few minutes before the blooming process, reasonably normal anthesis followed and a fair to good yield of dry pollen was easily obtained. These panicles usually bloomed little if any on subsequent days. *P. urvillei* responded least favorably to this treatment. When cut panicles were brought into the laboratory in the late afternoon, *P. malacophyllum* was the only one of these grasses that bloomed well and produced an abundance of pollen on the following morning. Panicles of *P. malacophyllum*, cut on the afternoons of rainy days and brought into the laboratory, responded as uncut heads in the field or greenhouse and failed to bloom during the next day.

Fruwirth (1916), studying the northern cultivated grasses, noted that the rate of blooming increases with temperature and stated "if there is not sufficient warmth available, blooming does not take place at all." He observed that the lack of light will delay blooming somewhat. He reported that although "high humidity" does delay the opening of the anthers it does not hinder blooming.

SUMMARY

During the four years 1938 to 1941, *Paspalum malacophyllum*, *P. dilatatum*, *P. urvillei* and common *P. notatum* produced panicles continuously from May or June until October. The Paraguay strain of *P. notatum* usually formed many panicles for about one month and relatively few thereafter. Generally *P. malacophyllum* bloomed an hour or more before sunrise, *P. urvillei* began to bloom before dawn but frequently had not finished by sunrise, Paraguay *P. notatum* bloomed about sunrise and *P. dilatatum* and common *P. notatum* bloomed from thirty minutes to an hour later.

Usually less than half of the florets on any one raceme bloomed during any twenty-four-hour period. Flowers in the terminal portion of the raceme and in the uppermost racemes in the panicle tended to bloom first. The outer spikelet in those species having paired spikelets (all but *P. notatum*) almost invariably bloomed first. Usually from fifteen to thirty minutes were required to complete the blooming process in any one floret.

Several controlled experiments indicated that anthesis in *P. urvillei*, *P. dilatatum* and *P. notatum* may be delayed several hours by excluding entire plants or just panicles from light. Within a rather narrow temperature range the rate of anthesis increased with temperature. These *Paspalums* either failed to flower or bloomed in a very abnormal man-

ner when air temperatures exceeded 80 degrees Fahrenheit. Mist, either natural or artificial, delayed dehiscence of the anthers and indicated that dehiscence is dependent upon desiccation.

P. malacophyllum was the only one of these grasses that failed to bloom on rainy days or on days immediately following rainy days, whether in the field or in the greenhouse.

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SPORELINGS AND VEGETATIVE REPRODUCTIVE STRUCTURES IN *MASTIGOLEJEUNEA AURICULATA*¹

Margaret Fulford

BOTH SPORELINGS and vegetative reproductive bodies from leaf cells were observed recently in the tropical and subtropical hepatic, *Mastigolejeunea auriculata*. Neither of these structures has been previously reported for the species, so that not only are the patterns of development in themselves of interest, but they afford the not too frequent opportunity of a comparison of development of young plants of a species from two points of origin, namely, the spore and the leaf cell. In addition to this, the vegetative reproductive structures develop from the apparently mature leaf cells which dedifferentiate before they divide to initiate the development of the new plant.

Evans (1902) has given a detailed description and figures of the mature plant so that only a very brief outline need be repeated here. The plants usually grow on banks in green to brownish depressed mats. The stems are often 2 cm. in length and the leafy shoots may be 2.5 mm. wide. The branches are of the *Lejeunea* type, are widely spreading and may be microphyllous or flagelliform. The leaves are ovate-oblong, up to 1 mm. in length and have a characteristic water sac (fig. 19). The underleaves are broadly orbicular, and may be up to 0.55 mm. long. The leaf cells of the apical portion average $24 \mu \times 16 \mu$. The walls are thin but have conspicuous trigones and intermediate thickenings.

THE SPORELING.—Sporelings were found in abundance in material collected at Troy, Jamaica, by Dr. Evans. At the time that the spores are shed they are

typical for spores formed in tetrads; that is to say, each has three flat, triangular surfaces and a bulging, rounded surface. The wall is densely echinate and hyaline (fig. 1), and the spore is green because of its chlorophyll content. With the increase in available moisture the spore becomes spherical. At about this time four cells can usually be distinguished, separated by walls more or less at right angles to one another within the original spore wall (fig. 2). Further divisions occur in one direction faster than in the others, and very soon the young sporeling is elongate ovoid and contains a fairly large number of cells (fig. 3 and 4). The echinate exospore is plainly visible and for a time forms an even covering over the whole cell mass. However, as these cells reach maturity they tend to become rounded on the free surface, so that in the leafy sporeling this basal portion which retains the echinate spore wall is irregular in outline and suggests a compact bunch of grapes (fig. 5 and 6). The multicellular body is usually about 60μ long when further cell divisions and growth cease, and the apical cell with three cutting faces is formed at one end. This in turn initiates the growth of the leafy shoot.

The leafy shoot first formed has only two rows of leaves. These are plane and ovate and represent the primary leaves, similar in character to those found in the sporelings of *Lopholejeunea*, *Stictolejeunea* and certain other of the leafy liverworts. As in the genera just mentioned there are no underleaves associated with the primary leaves. The formation of this type of leaf continues for some time, and it is assumed that under favorable conditions a more advanced stage of development is soon attained which will result in the formation of typical juvenile leaves and underleaves. This pattern of development of the sporeling conforms to the *Frullania* type (Fulford, 1942).

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The writer wishes to thank Dr. A. W. Evans of Yale University for his kind assistance, and the members of the Botany Department of Yale University and the New York Botanical Garden for facilities for study during the course of the work.

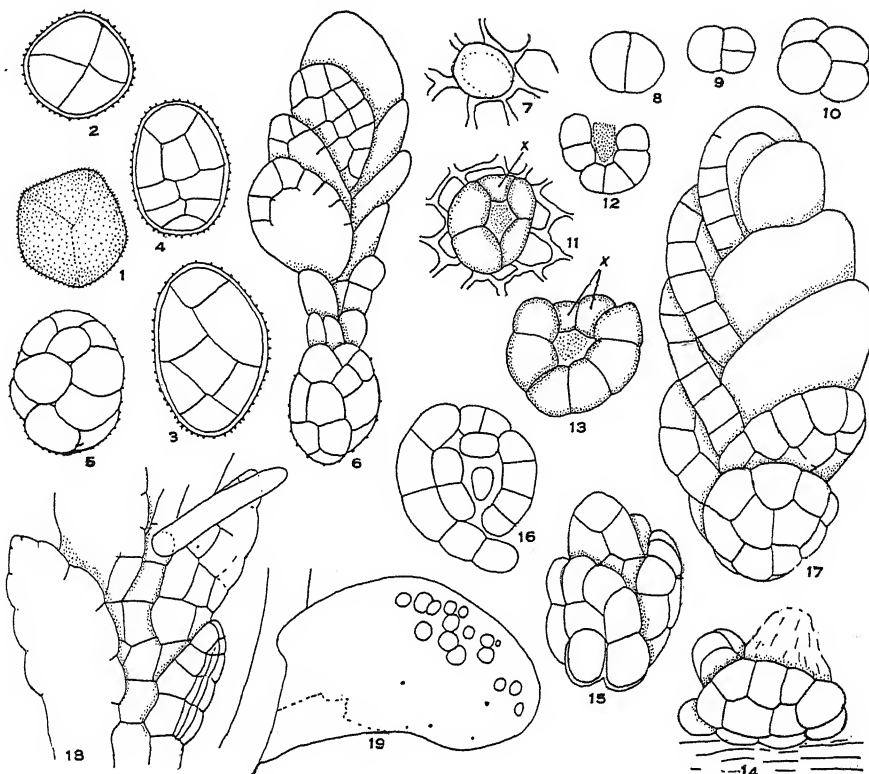


Fig. 1-19. *Mastigolejeunea auriculata*.—Fig. 1-6. Sporeling.—Fig. 1. A spore, $\times 400$.—Fig. 2-5. Stages in the development of the ovoid mass of cells within the exospore, $\times 300$.—Fig. 6. A sporeling with primary leaves, $\times 300$.—Fig. 7-18. Stages in the development of the vegetative reproductive shoot from a leaf cell, $\times 300$.—Fig. 7. A leaf cell protruding on the dorsal leaf surface.—Fig. 8. The division of this cell.—Fig. 9-10. Further divisions in the newly formed pair of cells; these develop into the first pair of leaves.—Fig. 11-13. Optical view of the early stages of the developing shoot through the activities of the apical cell with X the vertical segment.—Fig. 14-15. Very young shoots showing the first pair of leaves which developed from the divisions of the leaf cell.—Fig. 16. Optical view of an early stage of the shoot showing the segments cut off from the apical cell.—Fig. 17. A shoot with five pairs of primary leaves.—Fig. 18. Ventral view of a portion of a shoot to show the single row of cortical cells developed from the ventral segment and a rhizoid from one of the cells.—Fig. 19. A leaf of a mature plant showing the position of the vegetative reproductive bodies and the water sac, $\times 30$.

VEGETATIVE REPRODUCTION.—This phenomenon was observed in only two collections,² one from Costa Rica and the other from British Guiana, although the species is widespread in tropical and subtropical America from southern Florida and Mississippi through the West Indies, Mexico, and Central America to northern South America and is collected very frequently.

The vegetative reproductive structures are produced only on the leaves. These appear to be "normal" in every way and in no respect different from those of the rest of the plant or of other plants. They give no indication of being old, nor are they "eroded" or weathered as is the situation in *Bryopteris* (Fulford, 1941). The new plants usually develop in large numbers on the dorsal surface of a leaf (fig. 19), with a few on the ventral surface.

² The material from Costa Rica was collected by Professor Alfaro, at 'El General,' 720m., and is in the herbarium of Field Museum; that from British Guiana was collected by Professor P. W. Richards and is in the herbarium of Yale University.

Whether this is the result of an inherent polarity or of some external stimulus has not yet been demonstrated.

There is no evidence that the cells giving rise to new plants are not mature and like the other leaf cells. They have relatively thick walls with conspicuous trigones, but the cytoplasm and nuclei are difficult to demonstrate in dried material. The first step in the development of the new plant is apparently the dedifferentiation of the mature cell, and in this process the cytoplasm becomes granular and very conspicuous and the nucleus greatly enlarged.

Very soon a cell of this sort bulges on the dorsal surface of the leaf (fig. 7). The enlargement is followed by a transverse division at right angles to the long axis of the cell, so that two bulging cells are formed (fig. 8). Each of the two cells gives rise to a leaf of the primary type. Each cell divides into two cells through the formation of a wall at right angles to the first wall and also to the surface of the leaf (fig. 9 and 10). The two-celled stage is followed by additional divisions, until the characteristic pri-

mary leaf is formed (see fig. 9–17). During the development of this first pair of primary leaves the newly formed apical cell with three cutting faces, which will give rise to the rest of the shoot, is also active. It becomes evident at about the time that the first pair of leaves has reached the two-celled stage, but the details of its origin are so difficult to observe that the method of formation has not yet been determined.

The details of development of the ventral segments are not easily observed. It appears that they are cut off as soon as the apical cell becomes active, but that they form only stem tissue. It would seem that the first ventral segment cut off (*x*, fig. 11) divides to form two cortical cells (*x*, fig. 13), but the subsequent ventral segments give rise to only one cortical row and internal tissue, for the fully developed stem bearing primary leaves—always without underleaves—has only one row of cortical cells which are ventral in position (fig. 18). The cells of this row give rise to occasional rhizoids. The same situation is no doubt present in the leafy sporeling so long as primary leaves are formed, since here, also, no underleaves are present.

The leaves formed from the later lateral segments are similar to, but somewhat larger than, the first pair which were formed from the bulging leaf cells. They are plane and broadly ovate and are also of the primary type, with no indication of a water sac (fig. 17 and 18). Stems with as many as ten pairs of these leaves are not infrequent.

As in the case of the sporelings, no plants having juvenile leaves with the characteristic water sacs were found.

The new plants readily become separated from the parent leaf, and leave an empty space in the original cell network. Some traces of the first cross wall are often left behind.

There were many instances in which death of a leaf cell had occurred just after the change in the cell content was evident, or after the cell had enlarged or after a cross wall had formed, all of them recognized by a brown discoloration of the cell content, walls and trigones.

We have here another example of mature, differentiated cells which have retained their ability to divide. In addition to this, they are totipotent. The reason for the infrequency of expression of this ability and the external or internal stimuli which initiate it are not known. The development of the young leafy plant proceeds directly from the leaf cell without an intervening protonemal stage characteristic in some forms. In fact, the development is so direct that the two cells formed by the initial division of the leaf cell give rise to the first pair of leaves. Other examples of this nature in which the leafy shoot proceeds directly from the leaf cell have already been cited for this family by Evans (1906) and others.

The developmental pattern of the sporeling and the vegetative reproductive body are similar except for the fact that the initial stage of the sporeling, the ovoid cell mass, is absent in the latter. The general habit, the form and structure of the primary leaves, the absence of underleaves, and the stem are the same.

This same pattern of vegetative reproduction is present also in the South American *M. plicatiflora* and is no doubt characteristic of the genus.

SUMMARY

The pattern of development of the spore of *M. auriculata* is of the *Frullania* type; that is, an ovoid mass of cells is formed within the much stretched exospore.

The apical cell with three cutting faces develops at one end of this cell mass, and the leafy stem is formed through its activities.

The vegetative reproductive bodies are in the nature of leafy stems developed from individual cells of the mature leaf.

The mature leaf cells not only have the capacity to dedifferentiate and to divide; they are also totipotent.

No protonemal stage is formed, since the new leafy stem develops directly from the leaf cell; the two cells formed from the initial division of this leaf cell, through subsequent divisions, form the first pair of leaves.

The early leaves of both the sporeling and the vegetative reproductive structures are of the primary type, without water sacs, and often ten or more pairs are formed. Underleaves are not associated with these leaves.

During the stage when leaves of the primary type are formed the ventral segments give rise to one row of cortical cells and a portion of the medulla of the stem.

In this genus the pattern of development of a plant from a leaf cell follows that of the sporeling, except that it does not have the protonemal stage.

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THE THREE-DIMENSIONAL SHAPES OF UNDIFFERENTIATED CELLS IN THE PETIOLE OF ANGIOPTERIS EVECTA¹

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ONE of the most striking features of higher plants and animals is the physiological and structural diversity of their cellular tissues. Since the peculiarities of structure in given tissues are particularly revealed in the shapes of their cells, an analysis of the three-dimensional shapes of these cells should greatly increase the understanding of organization and differentiation in plants and animals alike.

In general, several types of investigation have a bearing on the problem of cell shapes. First, Lewis (1923, 1925, 1933a, and 1933b), Marvin (1939a), and Hulbary (1940) have concerned themselves with analyses of the actual three-dimensional shapes of mature cells in which, presumably, the forces determining shape have attained an equilibrium. Second, work with physical systems by Marvin (1939b) and Matzke (1939 and 1940a) has shown that pressure in lead shot and surface tension in soap bubbles produce shapes comparable to those of actual cells. In all cases the average number of contacts per cell or polyhedron in a central position approximated fourteen although the number of polyhedra having exactly fourteen facets was sometimes less than those having thirteen or fifteen. Thus the tetrakaidecahedron, or polyhedron with fourteen facets, is not necessarily achieved, though it might be said to represent the average. Third, studies of development such as those of Sinnott and Bloch (1939, 1941) indicate that plant cells undergo no drastic rearrangement in growth and, therefore, that the plane of cell division is often a primary factor in cell shape.

LITERATURE REVIEW.—A comprehensive review of the literature on cell shape has been given in a recent paper by Marvin (1939a) who has traced the interest in the three-dimensional shapes of cells from the time of Hooke and Grew in the seventeenth century through to the recent work of Lewis, Matzke, and others. Even more recently Matzke (1940b) has written a concise digest of the problem as it stands now. For this reason only the more pertinent and recent papers will be mentioned here.

The first actual investigation of the three-dimensional shapes of massed cells was made by Lewis (1923). By means of serial sections of elder pith he found that for a hundred cells the average number of contacts was 13.97 per cell. In a discussion of the problem from a biological standpoint Lewis concluded that, despite various modifying factors, elder pith cells approximated the orthic tetrakaidecahedron as described by Lord Kelvin in 1887. This figure is a polyhedron having fourteen facets, six squares and eight hexagons, and has several features which might be expected in cell masses. First, the figure has dihedral and trihedral angles but no tetra-

hedral angles which are less stable; second, it is very economical in its surface-volume relationships; and third, such figures will stack to fill space without interstices.

Lewis found no cell in the elder pith which conformed perfectly to the orthic tetrakaidecahedron nor did he find any in his later studies of human fat and tadpole precartilaginous cells. His observations showed that there were more pentagonal facets than either the square or the hexagonal facets expected if cells were orthic tetrakaidecahedra. He pointed out, however, that cell division and differences in cell size may modify the shape of a cell and, further, he showed how some of the cells found might be derived from the orthic tetrakaidecahedron. Thus his conclusion was that fundamentally cells have the three-dimensional form of the orthic tetrakaidecahedron.

Marvin (1939a) has recently reported a thorough investigation of a hundred pith cells of *Eupatorium*. Using a new technique (Marvin and Matzke, 1939) in which the cells were impregnated with wax and the resulting wax models separated, he was able to study each contact surface of every cell under a dissecting microscope. The average number of facets for the 100 cells was 13.36.

One of the most important features of the cell studies is the investigation of cell volumes. Started by Lewis, this line of investigation was greatly extended by Marvin (1939a) who made accurate, greatly enlarged, paper models of each of the 100 pith cells that he studied. The results of this work showed that smaller cells had fewer than fourteen contacts and larger cells more than fourteen contacts; that smaller facets had fewer edges than larger ones; and that the surface to volume relationships of cells closely approached the economy of surface found in the orthic tetrakaidecahedron or rhombic dodecahedron of equal volume. This last is a very important discovery for it shows that a high degree of economy of surface may be present in cells of undifferentiated tissues despite differences in volume and irregularities in shape of the cells.

Recent experimental work which seems to be closely related to cell shape has been reported by Marvin (1939b). Lead spheres of uniform diameter were subjected to compressive forces ranging up to 35,000 lbs. per sq. in., at which pressure all interstices were eliminated and an average of 14.17 contacts was established in the central shot. A lesser pressure (22,500 lbs.), in which some interstices remained, gave an average of 13.62 for the central shot.

Matzke (1939) studied compressed lead shot of different sizes, one having twice the diameter of the other. Various mixtures of the two showed that the smaller shot always had an average of less than fourteen facets. The averages of the central shot in

¹The author is deeply grateful to Professor Edwin B. Matzke for innumerable aids during the course of this research at Columbia University and its subsequent preparation for publication.

TABLE 1. Distribution of the 100 cells according to number of contacts per cell.

Number of contacts	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Number of cells	2	2	7	11	15	19	14	7	8	7	4	1	1	0	2

different experiments, small and large considered together, varied from 13.0 to 13.8 facets.

Further work by Matzke (1940a) on soap bubbles of uniform volume showed that a surface tension system also gave an average number (13.69) of contacts per polyhedron which approximated fourteen. Other striking results found in the study of soap bubbles were the large number of polyhedra with the same combinations of facets, the large number of pentagonal dodecahedra, and the greater proportion of pentagonal facets in general.

MATERIALS AND METHODS.—Since spermatophytes have been used in all previous botanical studies of cell shape, a pteridophyte, *Angiopteris evecta*,² was chosen in the present case in order to compare results in distant plant groups. The basal portion of an *Angiopteris* petiole, about 1½ inches in diameter, was fixed in 95 per cent ethyl alcohol. A cross section at this point showed a large number of vascular bundles loosely arranged in five to six rings within the ground tissue, or parenchyma. The parenchyma made up the bulk of the petiole and provided the cells reported on here.

Portions of the petiole were first cut into transverse sections about four to six cells in thickness. These sections were stained with safranin in 95 per cent alcohol, then gradually transferred to xylol, after which small rectangular blocks, approximately 0.5 mm. across, were cut longitudinally from the ground tissue between the bundles. The small blocks, containing from ten to thirty whole and unimpaired parenchymatous cells, were then placed in a mineral oil (Nujol). It was found possible to orient a block in any position in the mineral oil while studying the cells of the block under a binocular dissecting microscope. Thus any given facet of a cell could be seen in plane view when using a magnification of 100 diameters or more. In this way the number of contacts of each of a hundred cells was ascertained, and the number of edges on each contact was tabulated. Unless otherwise specified only contact facets have been considered as true cell facets. These hundred cells were not taken at random, but instead all the whole cells in a single block were studied and numbered in sequence before proceeding to another block. Six blocks were sufficient for the hundred cells. A schematic drawing showing the position of the cells was made of each block, so that it was possible to refer back to any given cell.

Shrinkage due to alcohol fixation may have affected slightly the size of intercellular spaces observed, but there is no evidence to indicate any significant alteration in the types of spaces, since these may be seen in the living tissue.

²The author wishes to acknowledge his appreciation to the New York Botanical Garden for material of *Angiopteris evecta*.

OBSERVATIONS.—In general the undifferentiated cells in the *Angiopteris* petiole are nearly isodiametric and tend to be irregularly arranged, unlike so many stem pith cells which lie in long continuous rows. Often, however, short, more or less irregular rows can be discerned. There is an indication of this in the photomicrograph of block 5 (fig. 1). The cells here vary considerably in size, and the tissue has some intercellular spaces. The two cells encircled in the photomicrograph (fig. 1) are also shown in figure 2.

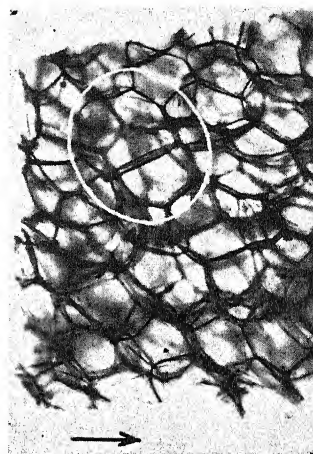


Fig. 1. Photomicrograph of a block of cells from the ground tissue of *Angiopteris* petiole. $\times 34$. The arrow indicates the approximate direction of the long axis.

A study of one hundred cells gave an average of 13.55 contacts. In *Angiopteris*, as can be seen in table 1, cells with thirteen contacts were the most frequent, being nineteen in number, but nearly as many, fifteen, had twelve contacts and fourteen of the hundred cells had exactly fourteen contacts. The range was from eight to twenty-two contacts but eighty-eight of the cells fell in the range between ten and seventeen. There was one break in the range of eight to twenty-two contacts, since no cells with twenty-one facets were found.

Volumes of cells were not measured, but the camera lucida drawings indicate (as found by Marvin, 1939a) that the cells with a lesser number of facets (fig. 3, 4, 5, 7) were smaller than the many-faceted cells (fig. 2, 8). On the whole, cells with twelve to fifteen facets were medium sized (fig. 6, 9, 10, 11).

In *Angiopteris* several of the cells studied were separated by intercellular spaces which quite possibly reduced the average of contact-facets per cell. One of these intercellular spaces is shown as a stippled area in figure 16, in which two cells barely miss contacting one another. Each of these intercellular

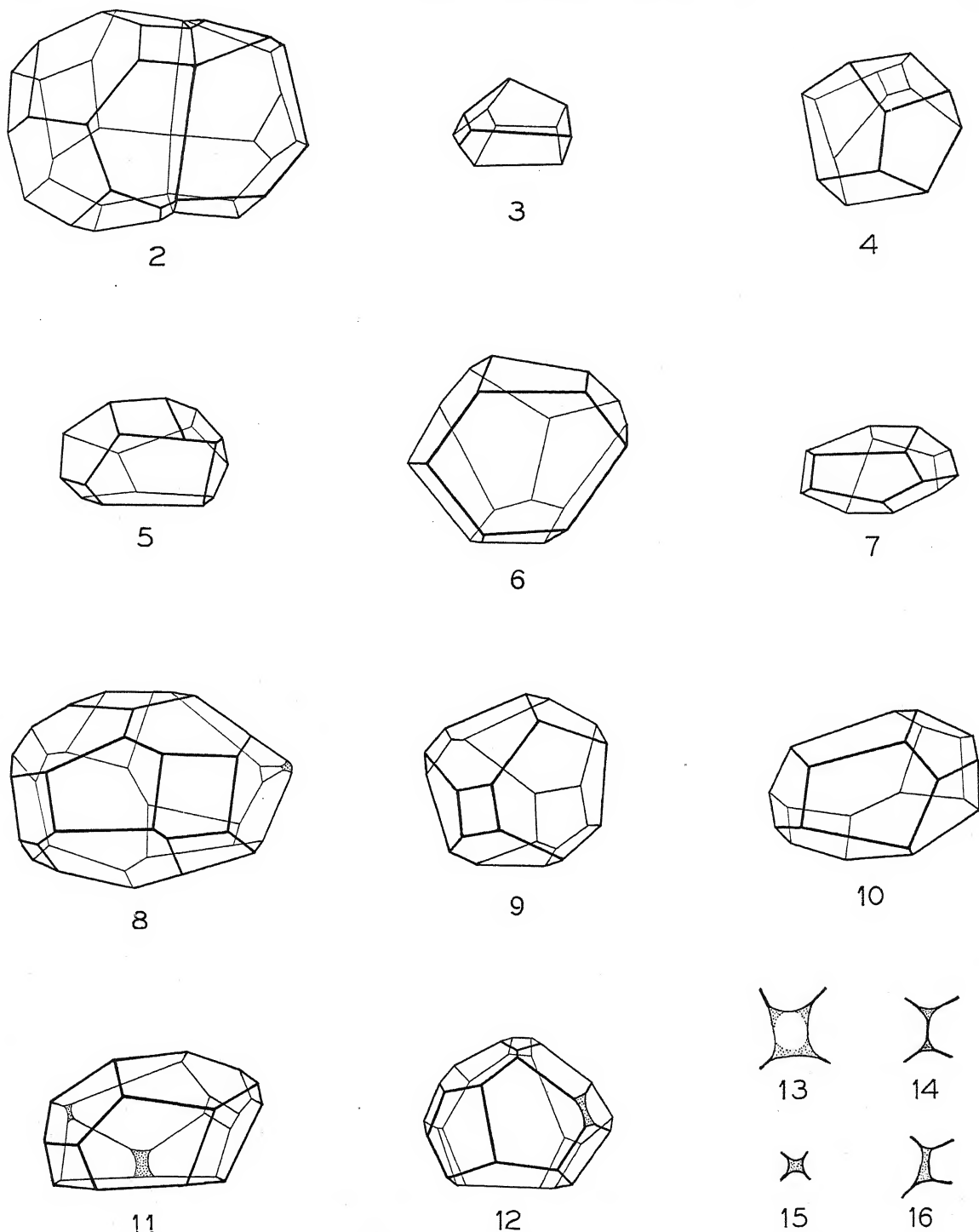


Fig. 2-16. Camera lucida drawings of some of the *Angiopteris* cells studied. $\times 150$. The number of contacts of each cell is followed by a specification of their shapes: T-triangular; Q-quadrangular; P-pentagonal; H-hexagonal; Hpt-heptagonal; O-octagonal.—Fig. 2. Cells 65 and 66. Cell 65, left, has 16 facets, 4 Q, 5 P, 6 H, and 1 Hpt. Cell 66, right, has 12 facets, 5 Q, 3 P, 3 H, and 1 Hpt.—Fig. 3. Cell 42 with 8 facets, 4 Q and 4 P.—Fig. 4. Cell 47 with 10 facets, 4 Q, 4 P, and 2 H.—Fig. 5. Cell 59 with 11 facets, 3 Q, 6 P, and 2 H.—Fig. 6. Cell 28 with 12 facets, 3 Q, 6 P, and 3 H.—Fig. 7. Cell 78 with 10 facets, 2 Q and 8 P.—Fig. 8. Cell 73 with 22 facets, 1 T, 5 Q, 5 P, 6 H, 4 Hpt, and 1 O.—Fig. 9. Cell 30 with 14 facets, 3 Q, 7 P, 3 H, and 1 Hpt.—Fig. 10. Cell 80 with 13 facets, 3 Q, 7 P, 2 H, and 1 Hpt.—Fig. 11. Cell 77 with 14 facets, 4 Q, 5 P, 4 H, and 1 Hpt.—Fig. 12. Cell 41 with 18 facets, 5 Q, 7 P, 2 H, 3 Hpt, and 1 O.—Fig. 13-14. Front (13) and side (14) view of a face of cell 30 showing an intercellular space at the angles and along the edges.—

TABLE 2. One hundred cells of *Angiopteris* petiole showing the number and kind of facets on each cell.

Facets per cell	Cell	No. of facets per No. of sides						Occur- rence	Facets per cell	Cell	No. of facets per No. of sides								Occur- rence
		3	4	5	6	7	8				3	4	5	6	7	8	9		
8	7, 42	0	4	4	0	0	0	2	15	97	0	1	10	4	0	0	0	1	
9	72	1	3	3	2	0	0	1	15	70	0	2	9	3	1	0	0	1	
9	38	1	4	1	3	0	0	1	15	49	0	3	8	2	2	0	0	1	
									15	82 ^a	0	3	8	4	0	0	0	1	
10	78	0	2	8	0	0	0	1	15	33 ^a	0	3	9	2	1	0	0	1	
10	19, 36, 47, 89	0	4	4	2	0	0	4	15	26	0	5	3	6	1	0	0	1	
10	45	0	5	3	1	1	0	1	15	27	0	5	6	1	2	1	0	1	
10	62	1	2	5	2	0	0	1	16	44	0	1	12	1	2	0	0	1	
11	9, 95	0	2	8	1	0	0	2	16	65, 67	0	4	5	6	1	0	0	2	
11	59, 81, 92	0	3	6	2	0	0	3	16	40	0	4	8	1	2	1	0	1	
11	23	0	4	4	3	0	0	1	16	64	0	5	4	6	0	1	0	1	
11	57, 83	0	5	2	4	0	0	2	16	14	0	5	5	4	1	1	0	1	
11	15	0	6	1	3	1	0	1	16	85 ^a	0	5	7	1	3	0	0	1	
11	74	1	1	7	2	0	0	1	16	20 ^b	0	6	6	2	2	0	0	1	
11	75	1	3	4	2	1	0	1	17	13	0	1	10	6	0	0	0	1	
12	8, 93	0	2	8	2	0	0	2	17	16	0	1	11	4	1	0	0	1	
12	28, 51, 56	0	3	6	3	0	0	3	17	50	0	2	11	2	1	1	0	1	
12	24, 71	0	3	7	1	1	0	2	17	87	0	3	8	4	2	0	0	1	
12	4, 55	0	4	4	4	0	0	2	17	76	0	3	9	3	1	1	0	1	
12	5, 6	0	4	5	2	1	0	2	17	61	0	4	7	3	3	0	0	1	
12	66, 84	0	5	3	3	1	0	2	17	1	0	6	5	3	2	0	1	1	
12	52 ^a	0	5	4	3	0	0	1	18	10	0	3	8	5	2	0	0	1	
12	32	1	3	4	3	1	0	1	18	91	0	3	9	3	3	0	0	1	
13	12	0	1	10	2	0	0	1	18	17	0	4	7	4	3	0	0	1	
13	3	0	2	8	3	0	0	1	18	41	0	5	7	2	3	1	0	1	
13	43, 60	0	2	9	1	1	0	2	19	31	0	3	9	4	3	0	0	1	
13	11, 18	0	3	6	4	0	0	2											
13	53, 80, 99	0	3	7	2	1	0	3	20	25	1	1	9	7	2	0	0	1	
13	37, 54, 98	0	4	4	5	0	0	3	22	88	0	4	8	7	2	1	0	1	
13	34, 94	0	4	5	3	1	0	2	22	73	1	5	5	6	4	1	0	1	
13	68, 69	0	5	3	4	1	0	2	Total all cells 1355 1-100		11 352 596 291 94 10 1 100								
13	29, 63	0	5	4	2	2	0	2											
13	79	1	4	4	1	3	0	1	Ave. 13.55		0.1 3.52 5.96 2.91 .94 0.1 .01 1.4								
14	100	0	2	8	4	0	0	1											
14	2, 22, 30, 35	0	3	7	3	1	0	4											
14	48, 58, 77	0	4	5	4	1	0	3											
14	39, 90	0	4	6	2	2	0	2											
14	96 ^a	0	4	6	4	0	0	1											
14	46	0	5	4	3	2	0	1											
14	86	0	5	5	2	1	1	1											
14	21 ^a	2	4	3	2	3	0	1											

^a Represents a tetrahedral angle. ^b Represents two tetrahedral angles.

spaces was surrounded by six cells and in one view showed two cells close together but not in contact (fig. 16), while the other view showed four cells surrounding a quadrilateral space (as fig. 15 with four cells shown at the sides while fifth and sixth cells—not shown—occupied positions above and below the plane of the drawing). In such cases it does not seem unlikely that the intercellular space has arisen from the separation of cells, and thus in a sense it represents a lost contact for each of the two proximal cells. For this reason, and for the sake of convenience, those regions at which one cell

barely failed to contact another cell through the intervention of an intercellular space have been termed "lost contacts."

In figure 11 the stippled areas represent two "lost contacts" on one cell. Other cells with lost contact areas are shown in figures 8 and 12. In general the larger air spaces causing lost contacts were associated with cells adjacent to one another but differing markedly in size.

In addition to the larger air spaces numerous smaller interstices were observed along the edges. Often they completely encircled a facet so that the

Fig. 15. An intercellular space at which 4 facets of cell 96 meet.—Fig. 16. A "lost contact" between cells 28 and 27. Compare this figure with figure 14.

contact appeared circular rather than polygonal (see figure 13 for face view, figure 14 for side view; the air space is indicated by stippling). It was possible to find all the intergradations in size of air spaces from minute "edge spaces" to the comparatively large ones which separated two or more cells. The "edge spaces" however, are not "lost contacts," since they do not affect the total number of contacts in a cell. In all, twenty-six "lost contacts" due to the presence of intercellular spaces were found in the hundred cells. If these twenty-six "lost contacts" were added to the total, the average number of facets would be increased to 13.81, which is considerably closer to fourteen.

Nearly all the data of this study have been included in table 2, which gives the number and kind of facets for each of the hundred cells. The cells are grouped according to number of facets and are on the same line if the combinations of facets are alike. The tabulation of the kind of facets found in the hundred cells shows that pentagons were most common by far. Of all the facets, 44 per cent were pentagons, 26 per cent were quadrangles, and 21 per cent were hexagons. An appreciable number of heptagons were found—7 per cent in all. These four kinds of facets, quadrangles, pentagons, hexagons, and heptagons, composed 98 per cent of all the facets. The remaining small number of the facets were triangles, octagons, and one nonagon.

At the bottom of table 2 may be seen the average per cell of each kind of facet, and thus the actual ratios of the kinds of facets. On the basis of approximate ratios a hypothetical average cell of *Angiopteris* would have four quadrangles, six pentagons, three hexagons, and one heptagon. It is obvious that this combination of facets would not fit together at all, since it gives an odd total number of sides, whereas in a polyhedron, such as a cell, each edge is shared by two facets always giving an even total number of sides.³ Marvin (1939a) found that *Eupatorium* pith gave a hypothetical average cell with four quadrangles, five pentagons, four hexagons, and one heptagon. This combination of facets is perfectly feasible mathematically, and three such cells were found in *Angiopteris* (table 2; see fig. 11).

The proportion of pentagonal facets found in *Angiopteris* was higher than that in other tissues reported heretofore—although in every case pentagons have been found to be the most common type. It is very interesting to note that Matzke (1940a, 1940b) observed that soap bubbles *en masse* actually showed more pentagonal facets than all other kinds of facets put together.

Reference to table 2 shows further that all the cells exhibit pentagonal and quadrangular facets, whereas hexagonal and other types of facets may be absent in certain cells. Three cells (table 2; see also fig. 3, 7) have only quadrangular and pentagonal facets. Needless to say such a predominance of quadrangles and pentagons (to the degree seen here) is

correlated with and tends to lower the average number of facets per cell in the type of system under consideration.

As expected, trihedral angles (2,294) were much more numerous than tetrahedral angles (8). With greater magnification, seven of the eight "tetrahedral angles" were seen in reality to be minute intercellular spaces at which four facets met (as in fig. 15), and only one of the "tetrahedral angles" actually approached geometric perfection. Nevertheless, since even these near-tetrahedral angles were so rare, 1 in 287, in undifferentiated tissue it seems obvious that the forces determining cell form in *Angiopteris* tend to avoid them. The appearance of small intercellular spaces at such angles is a mechanism whereby a more stable condition results (Lewis, 1933a).

Among the hundred cells studied certain combinations of facets were found to occur in two or more cells (see tables 2 and 3). Thus both of the two eight-faceted cells had the same combination, each having four quadrangular and four pentagonal facets. One of these cells is shown in figure 3. Of the seven ten-faceted cells found, four had four quadrangular, four pentagonal, and two hexagonal facets (see fig. 4). As may be seen in table 3, many other cases were found in which cells showed duplication in number and kinds of facets. It should be remembered, however, that this does not mean that the cells were exact duplicates of one another, inasmuch as they may vary in their different dimensions.

The average occurrence of any given facet combination (table 2) was 1.4 for the hundred cells.

TABLE 3. Combinations (of facets) occurring more than once in 100 cells.

Facets per cell	No. of cells	No. of facets per No. of sides			
		4	5	6	7
8	2	4	4	0	0
10	4	4	4	2	0
11	2	2	8	1	0
11	3	3	6	2	0
11	2	5	2	4	0
12	2	2	8	2	0
12	3	3	6	3	0
12	2	4	4	4	0
12	2	3	7	1	1
12	2	4	5	2	1
12	2	5	3	3	1
13	2	3	6	4	0
13	3	4	4	5	0
13	2	2	9	1	1
13	3	3	7	2	1
13	2	4	5	3	1
13	2	5	3	4	1
13	2	5	4	2	2
14	4	3	7	3	1
14	3	4	5	4	1
14	2	4	6	2	2
16	2	4	5	6	1

³ For a more detailed discussion of the mathematics and geometry of cell shapes see Lewis, 1933a, 1933b.

This does not necessarily indicate a pattern of cells in mass which was repeated 1.4 times in the group of one hundred cells. There was a more frequent occurrence of certain combinations, and thus of cell patterns or arrangements (see table 3), than of other combinations. Presumably those arrangements of cells which tended to comply more exactly with the laws of surface tension and pressure occurred more often than others. For example, those cells with trihedral angles were much more frequent than those with tetrahedral angles.

The figures in table 3 show that there were more cases of similar combinations in the thirteen-faceted cells, which were most numerous, than in any other. However, there were no more than three cells of any given combination, so that no single cell-form type is predominant. Among the fourteen-faceted cells, four cells had the following combination of facets: three quadrangles, seven pentagons, three hexagons, and one heptagon (see fig. 9). Again no single combination is outstanding. The cells illustrated in figures 2, 10, and 11 are additional examples of combinations which occurred twice or more.

Marvin (1939a) also noted in *Eupatorium* pith that many cells were alike in having the same number and kinds of facets. A comparison of the cell data presented here with Marvin's results shows that in many instances the same combination of facets was found in the cells of both tissues. In the hundred cells of *Eupatorium* pith each of sixty-three was similar to one or more other cells, while in the present study of *Angiopteris* each of fifty-three of the hundred cells was found to be similar to at least one other cell. Some of the combinations which occurred only once in Marvin's study matched some of those occurring only once in the present report, so that, if the data of the two studies are lumped together, it is seen that a total of 127 cells had combinations of facets similar to one or more other cells. It is interesting to note that with four exceptions these 127 cells had trihedral angles only, and all their facets were four to seven-sided, while among the remaining seventy-three cells (of the 200 total) seventeen had tetrahedral angles, and twenty-five had one or more of the very infrequent triangular, octagonal, or nonagonal facets. Thus only thirty-one cells, "typical" in having trihedral angles and

four to seven-edged facets only, had unduplicated combinations. Of these thirty-one cells one had twelve facets, one had thirteen facets, two had fourteen facets, and most of the remaining twenty-eight had fifteen or more facets each. Studies of larger numbers of cells would probably reveal duplications of combinations found in these thirty-one cells.

In view of total numbers in the two hundred cells, tetrahedral angles (one tetrahedral to 204 trihedral angles) and facets with a number of edges beyond the range of four to seven (one in seventy-six) were very infrequent, so that cells having these structures might be considered as exceptions to the usual tendencies. If these cells are omitted as atypical, then 79 per cent of the cells studied belong to the group in which certain combinations occurred twice or more.

DISCUSSION.—From the standpoint of cell shapes, comparison of the undifferentiated cells of a fern (*Angiopteris* leaf) with undifferentiated cells from widely separated sources as reported by other authors (table 4) has revealed no fundamental differences. Although nearly isodiametric cells seem essentially alike wherever they occur, no single ideal figure has been found to predominate in actual tissues. Reference to table 4 shows in every case a wide range in number of contacts per cell despite the fact that most cells, 81 per cent of the 450 cells reported, have from eleven to sixteen facets each. The average cell of the 450 total has 13.84 contacts and thus approaches a fourteen-faceted polyhedron more nearly than any other entire geometric figure. Despite this average it should be noted that there were as many cells with thirteen facets as with fourteen facets.

The loss of contacts due to the formation of intercellular spaces of the schizogenous type is to be expected as a common occurrence in plant tissues. However, Lewis found numerous edge spaces in elder pith (1923) which did not affect the average number of contacts. *Angiopteris* petiole tissue shows that relatively small intercellular spaces may lower the average number of contacts. In general the "lost contacts" (see fig. 8, 11, 12) are smaller than the actual contacts between two cells. This indicates that during development there is either a lesser wall growth in the region of an air space or a tendency

TABLE 4. Distribution of cells studied by various authors to date according to number of facets.

	Distribution of cells according to number of facets																	Ave. facets per cell
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Elder pith, 100 cells (Lewis, 1923) . . .	1	1	2	0	2	8	8	21	16	19	10	2	3	6	1	0	0	13.97
Human fat, 100 cells (Lewis, 1925) . .	0	0	0	2	4	5	15	13	20	15	17	4	2	2	1	0	0	14.01
Tadpole precartililage, 50 cells (Lewis, 1933a)	0	0	0	1	1	4	9	8	10	7	3	3	2	1	0	1	0	13.90
<i>Eupatorium</i> pith, 100 cells (Marvin, 1939a)	0	1	0	1	7	13	18	14	15	15	7	3	4	1	1	0	0	13.36
<i>Angiopteris</i> petiole, 100 cells	0	0	2	2	7	11	15	19	14	7	8	7	4	1	1	0	2	13.55
Total, 450 cells	1	2	4	6	21	41	65	75	75	63	45	19	15	11	4	1	2	13.84
Percentage	0.2	0.4	0.8	1.3	4.6	9.1	14.4	16.6	16.6	14.0	10.0	4.2	3.3	2.4	0.7	0.2	0.4	

for cells contacting one another with a small area to become separated.

The average number of contacts reported here for *Angiopteris* petiole, 13.55 for 100 cells, closely approaches the average 13.62 for lead shot when the shot was not compressed quite enough to eliminate all the interstices (Marvin, 1939b). Greater pressure on the lead shot eliminated all interstices and increased the average of contacts to 14.17. A similar increase in the average of contacts might be expected in *Angiopteris* cells if the air spaces could be eliminated.

Sinnott and Bloch (1941) have shown how the position of the new wall in cell division may affect cell shape. They found in certain grass roots that the position of the intercellular spaces was predetermined by the meeting of new cell walls to form four-rayed intersections. The air spaces always originated at the intersection. They further concluded that a pattern of cell division giving four-rayed intersections, as in certain grass roots, results in cells with ten facets each, and, if the walls are alternate in position avoiding four-rayed intersections, then an average of fourteen facets is to be expected.

In analyzing the shapes found in parenchymatous cells it is instructive to compare these shapes with those to be expected if a surface tension or interfacial film system is predominant. Surface films operating in cells would tend to give minimal surface area relative to volume, trihedral rather than tetrahedral angles, and a high proportion of pentagonal facets (see Matzke, 1940a). Marvin (1939a) has shown that, despite irregularities, cells do have an economy of surface area to volume relationship which compares favorably with both the rhombic dodecahedron and the orthic tetrakaidecahedron. All the cell shape studies (of compact undifferentiated tissue) have indicated a predominance of trihedral over tetrahedral angles. Matzke's study of soap bubbles (1940a) indicates that surface films may give many more pentagonal facets than any other kind. Pentagons are also much more frequent in cells than either quadrangular or hexagonal facets according to the present and previous reports. Thus ordinary parenchyma cells conform quite closely to the shapes expected of surface films.

The problem as to how, in special tissues, four-rayed intersections arise is not readily explained on the basis of surface films, however. Thus other forces such as turgor pressure and polarity—undoubtedly active in the process of differentiation—must be taken into account in proportion to their effect on cell shapes.

SUMMARY

The shapes of one hundred undifferentiated cells from the petiole base of *Angiopteris evecta* were studied by counting the number of contacts, or facets, per cell, and counting the number of sides of each contact. Instead of picking cells at random all the whole cells in six small rectangular blocks were studied.

The average number of contacts per cell was 13.55. In twenty-six cases cells barely missed contact through the intervention of intercellular spaces. Thus, in the absence of these intercellular spaces the average of 13.81 contacts per cell would be expected.

About 98 per cent of the facets of the cells studied were quadrangular (26 per cent), pentagonal (44 per cent), hexagonal (21 per cent), or heptagonal (7 per cent), while the range of facets was from triangular to nonagonal.

Nearly all of the 2,302 polyhedral angles were trihedral, only eight being classified as tetrahedral.

A number of cases were found in which more than one cell had the same number of each kind of facet. Thus four ten-faceted cells were found, each having four quadrangular, four pentagonal, and two hexagonal facets. In all, twenty-two combinations were found which occurred more than once in the hundred cells.

These observations and other data are discussed in relation to the theory that surface film forces predominate in determining cell shapes. Cell shapes do conform remarkably well with surface film expectations but do not achieve an ideal figure. Many factors other than surface film forces must be taken into account.

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POLAR AND APOLAR TRANSPORT OF AUXIN IN WOODY STEMS¹

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THE POLAR, basipetal transport of auxin in coleoptiles and young succulent stems of annual plants has been the subject of numerous investigations, but little study has been reported of auxins in stems of woody perennial plants. Such stems pass annually through active and inactive growth periods. Zimmermann (1936), Avery et al. (1937), Mirov (1941), Czaja (1934), van der Weij (1933) and Söding (1938) have shown the presence of auxins in woody stems. Van der Weij (1933) demonstrated polar movement of auxin in stems of *Elaeagnus angustifolia* Linn, and Michener (quoted by Went and Thimann, 1937) showed the same in *Salix*. The stems appear to have been used during the active period. Nothing seems to be known concerning auxin transport during the inactive or, as it is commonly termed, dormant season. Since changes occur during the dormant season which are important for subsequent growth, it seems desirable to learn something about the transport of auxin in dormant stems of plants which have a strong rest period. The purpose of this paper is to report studies on transport of auxin in pear and apple twigs during that part of dormancy which is termed the rest period, when the plants do not grow under otherwise favorable conditions of temperature, moisture and light. For purposes of comparison, certain experiments were carried out with newly-grown, non-resting tissues.

MATERIAL AND METHODS USED.—The resting twig sections used were obtained from one-year-old apple and pear trees kept in a warm greenhouse. The young, green sections were obtained from a group of trees similar in size and age to the resting trees, which, as a result of treatments with yeast extracts, opened several buds (Bennett and Skoog, 1938) and developed short shoots. The twigs were a few weeks old when used. The sections were 0.5 to 2 cm. long. The diameter of those from resting twigs was 3 to 4 mm., those from newly-grown twigs being somewhat smaller.

After cutting, the sections were floated on water for thirty to ninety minutes in order to wash the wound surfaces and to saturate the tissues with water. They were then dried with filter paper and sandwiched between two 1.5 per cent or 2.0 per cent agar blocks ($2 \times 2.7 \times 3.3$ mm.) either in the per-

pendicular or horizontal position, mostly the latter. The concentration of indoleacetic acid applied to the sections varied between 10 and 0.066 mgs. per liter of agar gel, and the amount applied per section varied between 0.300 and 0.002 micrograms (γ). The sandwiched sections were placed on glass slides inside a glass dish lined with moist paper. The tests were carried out at 25°C. except when otherwise stated. The sections were in contact with the agar blocks for periods varying from two to twenty-eight hours. The agar was then removed from the sections and tested for auxin content by the standard *Avena* test. In several instances the concentration of auxin in these blocks was too high to be measured directly, in which case a number of such agar blocks were placed in a test tube, a known amount of distilled water was added and allowed to stand in the dark at 0 to 2°C. overnight. The agar blocks were then placed on *Avena* coleoptiles, and their auxin content was determined after correction for the dilution factor. In a few cases, instead of soaking, the agar blocks were shaken in water at room temperature for several hours prior to placing them on the coleoptiles. In still other instances, the agar blocks were diluted with molten agar. When such dilutions were carried out, a set of agar blocks containing a known amount of indoleacetic acid, but previously not in contact with twig sections, was treated in the same manner and used as a check to correct for any loss of activity during the dilution process and during storage. All auxin data were based on eight to twenty-four *Avena* plants.

Agar blocks containing from three to five different concentrations of indoleacetic acid were tested with each set of samples. From the data thus obtained a curve of *Avena*-curvature vs. indoleacetic acid-concentration was constructed, from which the auxin content of the samples was determined by extrapolation or interpolation. This was done in order to correct for the daily variability in the sensitivity of *Avena* coleoptiles to indoleacetic acid, as well as for the deviation from proportionality of coleoptile curvature in relation to the concentration of this auxin. Some of the earlier data, however, were based on a single concentration of indoleacetic acid and strict proportionality was assumed between curvature and auxin concentration. Although such data are not accurate, they are valid for relative comparisons of treatments.

The extraction from twigs of both naturally occurring auxin and applied indoleacetic acid was car-

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ried out with 95 per cent methanol in soxhlet flasks. Extractions usually proceeded for twenty-four or forty-eight hours and were repeated until no further measurable amounts of auxin were yielded. The alcoholic extracts were evaporated to dryness on a steam bath assisted by an air blast. The dry residue was dissolved in 2 to 5 cc. of water on a steam bath for ten to twenty minutes, and the solution was mixed with an equal volume of 3 per cent agar. The agar gel was applied to the coleoptiles in blocks $2 \times 2.7 \times 1.7$ mm. = 14.3 mm.³ in size. One cc. of this gel corresponded to 0.5 to 1.5 gms. of fresh weight of extracted twig tissues. Water, acetone, ethyl-ether, 95 per cent ethanol, isopropyl alcohol, ethyl-acetate, ethyl-methyl-ketone and benzol also were found to extract auxin from resting twigs but were not used in the experiments reported.

TRANSPORT OF THE NATIVE AUXIN OF APPLE AND PEAR TWIGS.—The term native auxin in the present study applies to the naturally occurring auxin obtained from tissues by diffusion or by extraction with solvents. These extracts may possibly contain a mixture of several auxins. Free diffusible auxin was obtained from woody twigs by sandwiching sections 0.5 to 1 cm. long between agar blocks for two to twenty-eight hours. The agar blocks were then tested for auxin. These tests showed that young green twigs often (in six out of fourteen tests) contained appreciable amounts of auxin yielding curvatures of 1.5° to 11.4° . This auxin was transported basipetally, and no movement could be detected in the opposite direction (see table 1). Thus, the trans-

TABLE 1. *Auxin diffused into agar blocks from apical and basal ends of actively growing twig sections 1 cm. long. Diffusion for $2\frac{1}{2}$ to 3 hours at 25°C .*

Plant	Average <i>Avena</i> curvature caused by auxin diffused out from one section through	
	Apical end	Basal end
Hardy pear	0°	10.9°
	0	6.1
	0	7.0
Gravenstein apple	0	11.4
Grape vine	0	0.9
	0	0.8
Tobacco	0	5.6

port of the native auxin in young, actively-growing twigs is wholly or preponderantly polar. This does not necessarily exclude acropetal movement of auxin by way of the transpiration stream (see Hitchcock and Zimmerman, 1935, and Skoog, 1938). When the same tests were performed with the resting apple and pear twigs, no measurable or significant curvature of coleoptiles was obtained in the majority of cases. This was true for either the agar blocks placed at the apical end of the sections or those placed at the basal end. Occasionally a few blocks

in a set caused a negative curvature of 1 to 3° , but the average curvature for these sets was below 1° and not significantly different from 0° .

Although resting twigs usually do not contain measurable amounts of diffusible auxin, they nevertheless possess the mechanism for transporting auxin polarly, e.g., basipetally. This is shown by the following experiment. Agar blocks were applied to sections of young, actively-growing Gravenstein apple twigs, and the blocks now containing free diffusible auxin were applied to sections of resting Gravenstein twigs. Half of the auxin-loaded blocks were applied to the apical ends of the sections and the remaining applied to the basal ends. At the same time plain agar blocks were placed at the opposite ends of all sections to collect any transported auxin. After four hours the auxin content of the agar blocks was determined. The results were as follows:

	Average curvature
Agar blocks at basal end of sections—auxin applied to apical end.....	5.2°
Agar blocks at apical end of sections—auxin applied to basal end.....	0°
Agar blocks containing auxin but not applied to sections	4.6°
Check—Plain agar blocks on both ends of sections	0°

The auxin in the blocks applied at the apical end of the twigs was transported basipetally, but no detectable amount was transported in the opposite direction. This is not necessarily a proof of the complete absence of acropetal, apolar, movement of native auxin in the twig sections, since, when auxin was applied to the basal ends of the sections, an apolar transport by diffusion must have taken place through the dead lumina of the conducting system.

TRANSPORT OF INDOLEACETIC ACID.—Experiments similar to those described above were performed with agar blocks containing indoleacetic acid instead of native auxin. The results are presented in table 2.

It must be borne in mind, when evaluating the data in table 2, and other tables, that average *Avena* curvature of less than 1° can hardly be considered significant. Such values are designated by 0 in table 2. The sensitivity of the *Avena* test was such that 0 to 0.5° of negative curvature corresponded to approximately 0.01 p.p.m. indoleacetic acid in the agar blocks applied to the coleoptile. Therefore a "0" curvature in table 2 does not necessarily indicate that no indoleacetic acid was transported; but rather that if any was transported it was not in excess of approximately 0.003 γ per twig section.

Basipetal transport was indicated in twenty-six tests, of which only five showed significant transport in the opposite direction. In the latter tests the amount of auxin transported basipetally was three to thirteen times as large as that transported acropetally. It is reasonable to assume that the apolar auxin transport would be equal in both directions under the same conditions, because it is governed chiefly by diffusion. In the above-mentioned tests the apolar

TABLE 2. Basipetal and acropetal movement of indoleacetic acid through sections of resting apple and pear twigs. Sections were 5 mm. long, and transport lasted from two to sixteen hours at 25°C. Indoleacetic acid was applied in agar blocks either at the basal or the apical ends of the section.

Plant	Concentration of applied auxin in agar blocks	Average <i>Avena</i> curvature caused by indoleacetic acid transported through twig sections		Polar transport	Apolar transport Polar transport
		Basipetally	Acropetally		
	p.p.m.				
Newtown apple	0.08	0°	0°
Rome Beauty apple	0.10	0	0
	0.10	0	0
	0.10	0	0
	0.13	0	0
	1.00	1.9	0	1.9°	...
	1.00	10.0	0	10.0	...
	2.00	6.0	0	6.0	...
	2.00	13.0	0	13.0	...
	2.00	13.0	2.2	10.8	4.9
	4.00	1.0	0	1.0	...
Hardy pear	4.00	5.7	0	5.7	...
	0.08	0	0
	0.10	1.8	0	1.8	...
	0.10	5.0	1.2	3.8	3.2
	0.10	2.5	0.0	2.5	...
	0.10	4.2	1.0	3.2	3.2
	0.2	0	0
	0.4	0.4	0.8	0.4	0.5
	0.4	1.4	0	1.4	...
	0.4	2.6	0	2.6	...
	0.8	3.5	0	3.5	...
	0.8	5.0	0	5.0	...
	0.8	2.0	0	2.0	...
	0.8	0.5	0
	0.8	1.8	0	1.8	...
	1.33	7.9	0	7.9	...
	2.00	11.7	0	11.7	...
	4.00	12.1	0	12.1	...
	4.00	4.3	0	4.3	...
	4.00	7.2	0	7.2	...
	4.00	12.7	1.0	11.7	11.7
	10.00	12.3	0	12.3	...
	10.00	11.5	3.9	7.6	1.9

component of the basipetal transport could not be measured directly, but was presumably equal in magnitude to the acropetal transport. Hence the difference between the total basipetal transport and the acropetal transport represented the polar transport. The ratio of the polar to the apolar transport in these experiments varied between 2 and 12. It is impossible to evaluate the ratio in those tests in which no significant curvature was obtained for the acropetal transport on account of the limit of the sensitivity of the *Avena* test. It is very likely that in these cases the ratio might be larger than that given above. But the five above-mentioned cases suffice to show that, at least in some instances, the basipetal transport of auxin in resting woody twigs may be no more than three to six times as large as the acropetal transport.

Similar results were obtained when the following experiment was carried out. Resting twigs were cut into 5 or 10 mm. long sections, immersed in indoleacetic acid solutions for two days at 2 to 5°C. The sections were then removed from these solutions, dried with filter paper, and agar blocks applied to both apical and basal ends. The whole was then placed at 25°C. in a saturated atmosphere for two to six hours, the agar blocks removed and tested for indoleacetic acid. The results of these tests are presented in table 3. Here, too, it is shown that when the twigs were immersed in solutions of low concentrations (0.1 and 0.133 p.p.m., respectively) no appreciable amounts of indoleacetic acid diffused out either through the apical or the basal ends. With higher concentration (0.2 and 0.4 p.p.m.) substantial amounts of indoleacetic acid passed out through

TABLE 3. *Indoleacetic acid diffused out of apical and basal ends of resting twig sections immersed for forty-eight hours in solutions of indoleacetic acid in cold storage. Period of diffusion two to four hours. Sections 5 or 10 mm. long.*

Plant	Concentration of indole- acetic acid solution	Average <i>Avena</i> curvature caused by indoleacetic acid diffused out from one section through		Polar transport
		Apical end	Basal end	
	p.p.m.			
Hardy pear	0.2	0°	7.8°	7.8°
	0.2	0	7.8	7.8
	0.2	1.0	6.1	5.1
	0.4	1.6	12.3	10.7
	0.8	7.0	11.6	4.6
	4.0	12.4	11.0	-1.4
Rome Beauty apple	0.10	0	0	...
	0.13	0	0	...
	0.2	0	6.8	6.8
	0.8	6.3	11.9	5.6
	4.0	11.7	8.7	-3.0

the basal ends of the twigs, but little or none out of the apical ends. Finally with still higher concentrations (0.8 and 4.0 p.p.m.) copious diffusion of the auxin took place from both the apical and the basal ends; indeed, when the initial concentration of the indoleacetic acid solution in which the twig sections were immersed was 4.0 p.p.m., the amount of indoleacetic acid diffusing out through the apical ends was even slightly in excess of that obtained at the basal ends. At least part and perhaps all of the auxin diffused out may have come from the conducting system of the twigs.

Thus, unlike the native auxin in which case only basipetal movement could be detected, the transport of applied indoleacetic acid in resting pear and apple twigs took place both basipetally and acropetally. Although the basipetal transport is faster than the acropetal one, when the auxin is applied at low concentrations, it is not necessarily so at high concentrations. The ratio of the amounts of indoleacetic acid carried basipetally to that transported acropetally may be dependent not only on the twig material used but also on time of diffusion and auxin concentration. The data on hand, however, are inadequate to establish any possible quantitative relations between these factors. Whether or not such relations exist in the case of the native auxin could not be ascertained, because all of the tests carried out with this auxin involved low concentrations of this substance, in which case only the basipetal transport could be detected.

Acropetal transport of indoleacetic acid in coleoptiles and pea shoots previously reported by various investigators was criticized (Went and Thimann, 1937, p. 100-101) on the ground that such a transport was observed only when the concentration of applied auxin was very large (a hundred or thousand-fold of that existing in the tissues), and that, therefore, such findings had no bearing on the normal transport of auxin. It is shown in table 2 that

appreciable acropetal transport of indoleacetic acid was observed in five cases, of which two occurred at a concentration of 0.1 p.p.m. of indoleacetic acid in the applied agar blocks, e.g. 0.0038 γ auxin applied per twig section, which amount yields a coleoptile curvature of approximately 14°. Actively-growing twig sections of the same size excreted native auxin during two to four hours in amounts which yielded curvatures up to 11.4° per section; this corresponds to approximately 0.002 γ . Mirov (1941) working with sections of pine twigs and using a similar technique observed curvatures as high as 18°, corresponding to 0.003 to 0.004 γ indoleacetic acid per section. Czaja (1934), employing an auxin test less sensitive than the standard *Avena* test, obtained from apical internodes of etiolated shoots of woody plants curvatures up to 23° per 5 mm. long sections. Hence, the amount of indoleacetic acid applied in the above-mentioned experiments in which acropetal transport of indoleacetic acid was observed is decidedly within the range of "physiological amounts"² of auxin, insofar as it may be compared with the native auxin on the basis of degrees of *Avena* curvatures.

It is evident from table 2, however, that when indoleacetic acid was applied in physiological amounts, e.g., in concentrations of about 0.2 p.p.m. or less, often no transport of auxin through the twig sections could be detected in either direction. It was found that under such circumstances a substantial part of the applied auxin was lost from the agar block containing it. This took place both at the apical and the basal ends of the twigs. Part of this auxin could be recovered from the sections by extraction with 95 per cent methanol, but the recovery was short of 100 per cent. In at least one instance, however, appreciable amounts of indole-

² The term "physiological amounts" of auxin, as used in this paper, applies to the amount of the naturally occurring auxin in plant tissues, obtained by diffusion from such tissues.

^a Values in this test were corrected for acropetal transport which amounted to 1.3–1.6° per section.

ing Hardy pear twigs, which yielded no diffusible native auxin. Twig sections 5 mm. long were divided into two lots. Agar blocks containing indoleacetic acid were placed at the apical ends and plain agar blocks at the basal ends of one lot of sections. The sections of the other lot were separated into bark and wood with as little injury to the bark as possible. The bark was rolled and held together by rubber bands. The wood and bark were then treated separately with agar blocks in the same manner as the intact sections; after a period of diffusion of four or five hours at 25°C. in a moisture-saturated atmosphere, the agar blocks at the basal ends were removed and examined for auxin. In another series of tests, resting Hardy twigs were cut into 5 mm. long sections, which were immersed for two days at 0°C. in solutions of indoleacetic acid of varying concentrations. The sections were then removed, dried with filter paper, divided into two lots and treated like those of the previous tests. The results of the two series of tests are presented in table 4. It was found that, after immersion in 4.0 p.p.m. indoleacetic acid, significant amounts of the auxin diffused through the apical ends. This diffusion represents the non-polar component of the transport; hence, the values of the basal agar blocks were corrected for this non-polar transport, and the corrected figures are presented in the last column of table 4. No measurable diffusion from apical ends occurred with the lower concentrations of indoleacetic acid.

It is evident from this table that: (a) basipetal transport took place in both bark and wood in three out of four sets of twigs. The reason for the lack of significant auxin transport in the sets of column 3 when bark and wood were examined separately is not known; (b) in all tests the amount transported through the wood was larger than that transported through the bark, although the cross section area of bark and wood was nearly equal. This may be at least partly due to the more extensive injury sustained by the bark during the process of peeling and subsequent handling, despite all precautions taken; (c) the sum of auxin transported through the separated bark and wood is smaller than that transported through the intact twig sections. This may also be the effect of injury; (d) it is also clear that the cambium cannot be the sole path of transport, if it transports auxin at all, since the wood sections which transported a fair fraction of the auxin were scraped thoroughly and were, no doubt, free of cambium.

These findings are contradictory to those of Cooper (1936) who concluded from the rooting response of ringed citrus cuttings to applications of indoleacetic acid that the latter moves only, or mostly, in the phloem. Cooper's data, however, are indirect and may be interpreted as showing that "rhizocaline," rather than heteroauxin indoleacetic acid, is not transported in the xylem. The present results are also at variance with the observations of Söding (1938), who found that the diffusate from bark or wood from twig sections, which had the cambium

scraped off, or water extractions of scrapings from wood and bark produced little or no curvature of *Avena* coleoptiles, while cambium scrapings gave pronounced curvatures. He concluded that cambium (and possibly also the adjacent young xylem or phloem tissues) is the sole path of transport of auxin in woody stems. It is not necessarily true, however, that the presence of a substance in a given tissue indicates that it is transported through that tissue. It might be merely accumulated there. Further, Söding states that the auxin he obtained from cambium scrapings represented a water extract of the tissue, rather than free diffusible auxin. It might have included chiefly auxin liberated from crushed or dead cells.

EFFECT OF STEAMING AND ETHERIZATION ON TRANSPORT OF INDOLEACETIC ACID.—Polar transport in coleoptiles is associated with activity of living cells. It is reversibly inhibited by exposure of coleoptile sections to ether (van der Weij, 1934, Clark, 1938). The effect of etherization and killing of woody sections by boiling or steaming for twenty to forty minutes upon transport of indoleacetic acid was determined as follows. Twig sections, approximately 5 mm. long, were placed in a moist chamber containing an open vessel of ether for sixty to ninety minutes at 25°C. Agar blocks containing indoleacetic acid were then applied to either the apical or basal ends and plain agar blocks to the opposite ends. After additional exposures for two to five hours to saturated ether vapor at 25°C. the originally plain agar blocks were examined for indoleacetic acid. The results are presented in table 5. In four of the six etherization tests basipetal transport substantially exceeded acropetal transport. In one it was completely inhibited and in another, for reasons unknown, acropetal exceeded basipetal movement. The partial pressure of ether in the moist chamber was approximately three times as high as that found by van der Weij (1934) to completely inhibit polar transport in coleoptile sections. The incomplete inhibition of polar transport in woody twigs as contrasted with that of coleoptiles may be due to incomplete permeation of the twig sections with ether, although some loss in "semipermeability" of twig tissues was indicated by diffusion of brown pigments from the twigs into the agar blocks. It is not known to what extent the injury by ether was reversible.

Steaming or boiling, however, completely inhibited polar transport in five out of six tests, and only in one test a slight, and perhaps an insignificant, portion of the polar transport persisted. But neither etherization nor steaming had consistently altered acropetal (apolar) transport as compared with the corresponding transport through non-treated sections.

The data in table 5 indicate that polar transport in resting pear and apple twigs is, like that in coleoptiles, carried out by living cells, and by its very polar nature cannot be ascribed to free diffusion.

THE MECHANISM OF APOLAR TRANSPORT OF INDOLEACETIC ACID IN TWIGS.—Diffusion may play only a

TABLE 5. *Effect of etherization, steaming and boiling of apple and pear twig sections on polar and apolar transport of indoleacetic acid.*

Plant	Treatment of twigs	Concentration of indoleacetic acid in applied agar blocks	Average <i>Avena</i> curvature caused by indoleacetic acid diffused out from one section through		Polar transport
			Apical end	Basal end	
		p.p.m.			
Rome Beauty apple	Etherized	0.067	0.9°	3.3°	2.4°
	Boiled	0.067	0	0	0
Hardy pear	Not treated	0.80	0	5.0	5.0
	Etherized	0.80	0	0	0
	Steamed	0.80	0	0	0
Hardy pear	Not treated	1.33	0	7.9	7.9
	Etherized	1.33	0.6	6.0	5.4
	Steamed	1.33	0.7	2.5	1.8
Hardy pear	Not treated	2.0	0.3	11.7	11.4
	Etherized	2.0	4.1	1.0	-3.1
	Steamed	2.0	2.1	0	2.1
Hardy pear	Not treated	4.00	1.0	12.7	11.7
	Etherized	4.00	0	6.0	6.0
	Steamed	4.00	2.0	2.5	-0.5
Hardy pear	Not treated	10.00	3.9	11.5	7.6
	Etherized	10.00	3.1	9.1	6.0
	Steamed	10.00	1.7	1.0	-0.7

minor role in the movement of auxin inside living cells or across their membranes but must be an important factor in the transport of auxin through lumina of dead cells and cell walls. There is, therefore, good reason to assume that the apolar auxin transport in woody twigs is governed by diffusion. On the other hand, there is evidence to show that apolar transport of indoleacetic acid in woody twigs is not solely a process of *free* diffusion, but that other factors operate to modify this process. This is demonstrated by the following experiment.

Three grams of resting Hardy twigs were cut into 2mm. long sections and soaked overnight at 13°C. in 10 cc. of 0.24 p.p.m. indoleacetic acid. The sections were then removed from the solution and the indoleacetic acid, extracted in a soxhlet by 95 per cent methanol, was determined, as well as the residual concentration of the auxin in the solution. The latter was 0.15 p.p.m. The amount of indoleacetic acid extracted from the sections was, after correction for the native auxin extracted from sections soaked in distilled water, 0.27 p.p.m. expressed on fresh weight basis or 0.52 p.p.m. on the basis of the water content of the sections. If it be assumed that all the auxin absorbed by the tissues was present in them as free indoleacetic acid, then the concentration of auxin in these tissues was approximately 2 to 3½ times larger than in the surrounding solution, which is incompatible with free diffusion. The accumulation of indoleacetic acid in the sections can be explained, however, on the assumption that most of the auxin taken up by the tissues was re-

tained in living cells or was otherwise combined or adsorbed.

This assumption was substantiated by results obtained in another experiment in which a solution containing two parts per million of indoleacetic acid was passed from the apical ends through three-internode lengths of resting Hardy twig sections. Suction was applied at the basal end for 1½ to 2½ hours. Five cc. of solution, equivalent to 10 γ of indoleacetic acid, were forced through each section. Distilled water was then passed through the twigs in order to flush the conducting system free of auxin. The twigs were then extracted with 95 per cent methanol in soxhlet flasks until no more indoleacetic acid was obtained. The indoleacetic acid in these extracts, after correction for the native auxin extracted from check twigs injected with distilled water only, represents the amount of indoleacetic acid retained by the twigs either through adsorption, cell absorption, or by other means. Another set of twigs was steamed for thirty minutes prior to infiltration with indoleacetic acid, but otherwise was treated in the manner described above. The results were as follows. The auxin extracted per gram of tissue—reckoned as indoleacetic acid—was 0.04 γ for the twigs treated with water only, 0.40 γ for the steamed (dead) and 2.1 γ for the non-steamed (living) sections. Thus, both dead and living twigs possess a high capacity for retention of indoleacetic acid, but that of the living twig is several times larger than that of dead twigs.

In dead tissues accumulation of *free* indoleacetic acid in cells against a concentration gradient, as may

occur in living tissues, is ruled out. The retention of indoleacetic acid by steamed twigs must then be due in large measure to fixation of auxin in a water insoluble form. Skoog and Thimann's data (1940) suggest that proteins may act as auxin binders. Not all the indoleacetic acid penetrating the sections is necessarily fixed, however, and twigs to which this acid is applied through agar blocks, or by soaking in indoleacetic acid solutions, yield some diffusible auxin, provided enough of it is added, as indicated in tables 2 to 5.

The facts presented in this study can be explained by assuming that resting and possibly also actively growing twigs can absorb and transport indoleacetic acid apolarly, that is, both basipetally and acropetally, the direction of transport being determined by the concentration gradient. This is accomplished mainly by diffusion, which is, however, modified by fixation of the auxin along its translocation path. In addition to this mode of translocation there exists also a mechanism transporting indoleacetic acid in the basipetal direction only. This is the polar transport. Under the conditions studied both modes of transport operated simultaneously, the polar movement of the auxin being superimposed on that of diffusion.

In the experiments reported in this study polar transport was represented by the difference between the indoleacetic acid transported or absorbed basipetally and that absorbed or transported acropetally. Unlike coleoptiles apolar transport of this auxin in resting woody twigs can account for a substantial fraction of the total indoleacetic acid transported, when the latter is applied in concentrations of the order of 1 to 10 p.p.m. and may even obscure the polar transport (see table 3). The difference in behavior of coleoptiles and woody twigs may be at least partially accounted for by the difference in their anatomical structures; woody twigs contain many dead cells through which transport by diffusion can operate, while polar transport in coleoptiles, as well as in woody twigs, is carried out by living cells, in which *Avena* coleoptiles are proportionately richer than woody twigs.

While resting apple and pear twigs possess the capacity to transport indoleacetic acid acropetally and apolarly, in the experiments reported in this study transport was observed under artificial conditions. It is not known to what extent apolar translocation is a normal component of auxin transport in woody twigs.

SUMMARY

Non-resting, woody apple and pear twigs often contain free-diffusible auxin, while resting twigs rarely yield diffusible auxin in measurable amounts, although they contain appreciable amounts of auxin extractable with water, ether, acetone, methanol, ethanol, ethyl-acetate and benzol.

In non-resting twigs the movement of the native free-diffusible auxin in the direction of the long axis of the twig is mostly or entirely polar and basipetal.

In resting twigs native auxin externally applied by means of agar blocks was transported basipetally but no measurable transport could be detected in the opposite direction.

With indoleacetic acid applied by agar blocks in widely varying concentrations, basipetal transport was evident in twenty-six of thirty-four tests, while significant acropetal transport was indicated in six of the thirty-four tests. In two tests acropetal transport was observed when indoleacetic acid was applied in physiological amounts (0.1 p.p.m.). Basipetal transport of indoleacetic acid is more rapid than acropetal transport, but in five of the six above-mentioned tests the amount of the auxin transported basipetally was only 1 to 6 times as large as that transported acropetally, and in one instance it was of the same order of magnitude as basipetal translocation. This ratio is considerably smaller than that reported for *Avena* coleoptiles, for similar concentrations of applied indoleacetic acid.

Translocation of indoleacetic acid in sections of resting apple and pear twigs involves two simultaneously operating processes, namely polar transport and apolar transport. The polar transport operates basipetally only and is presumably carried out by living cells, being completely inhibited by steaming and partially inhibited by exposure to saturated ether vapor at 25°C. for several hours. The polar transport which operates both basipetally and acropetally is probably a diffusion process through the lumina of dead cells and through cell walls, but this process is modified by "fixation" or destruction of auxin along its diffusion path. A substantial amount, and sometimes all, of the "fixed" auxin can be recovered from the twigs by extraction with 95 per cent methanol.

Basipetal transport of indoleacetic acid and of the native auxin of pear and apple twigs takes place in both bark and wood. Available evidence does not indicate that cambium accounts for the bulk of basipetal transport of auxin, if it is involved in it at all.

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CHROMOSOMAL EFFECTS OF LOW X-RAY DOSES ON FIVE-DAY TRADESCANTIA MICROSPORES¹

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PART I. EXPERIMENTAL²

ROENTGEN RAY effects on *Tradescantia* chromosomes have received increasing attention among geneticists since Sax (1938, 1939) demonstrated the possibility of obtaining quantitative results. In spite of the rapidly growing number of contributions which have appeared since (Darlington and Upcott, 1940; Fabergé, 1940a and b; Giles, 1940; Rick, 1940; Sax and Swanson, 1941; Newcombe, 1942a and b, and a summary by Sax, 1941), the literature still contains many contradictions, as well as a wealth of non-comparable data, which, as a whole, have not yielded to the scrutiny of a mathematical investigation. In the face of these shortcomings it was considered worthwhile to determine critically the limitations of the experimental method employed, as well as to relieve a somewhat confused situation by investigating rather closely two classes of aberrations only, and by restricting radiation dosage to the low range.

The present paper endeavors to present: (a) the relationship between effect and radiation intensity at constant dosage covering a range of 1 to 1000 (hereafter referred to as intensity experiment); (b) a series of experiments obtained from different x-ray apparatus and scored by different observers; these experiments were designed to show the variation of effect with radiation dose (to be called dosage experiments); (c) the statistical basis of sample size and of error estimates; (d) a unified mathematical interpretation which covers the dosage and intensity data of this paper as well as comparable data of the literature; and (e) morphological deductions and general predictions based upon the foregoing analysis.

MATERIAL AND METHODS.—Plants of a clone of *Tradescantia canaliculata* Raf. (*T. reflexa* Raf.) were grown in pots at Geneva, New York, using anthers from uncut plants for observation. Plants of

the same clone were grown in the garden of the Carnegie Institution of Washington at Cold Spring Harbor, New York. All data derived from the latter plants are from cut flower heads taken as follows: Inflorescences with six-inch stems were taken from the garden a short time (at most two to three hours) before irradiation, labelled with serial numbers and distributed at random in groups of twelve into four sample vials and one control, all vials being filled with spring water. The material was rayed during interphase previous to the onset of nuclear enlargement (cf. Sax, 1938). During irradiation the plants and cuttings were kept fresh by means of wet toweling and returned to the vials immediately after irradiation and kept in this manner until each experiment was completed. In all cases smear preparations were made on the fourth and fifth day. The material was fixed on the slide with 3:1 alcohol acetic acid, stained with aceto carmine and mounted in Zirkle's fluid. The dosage data were taken by separate and, in part, independent observers from radiation supplied by different x-ray machines. Variation in the climatic environment, the use of two distinct clones of plants, and a comparison between cut and uncut flower heads did not materially alter the results under investigation. Certain inconsistencies in the literature, as well as in the present data, are not believed to be due to any one of the three factors mentioned above.

The data are tabulated according to investigators, and time and place of the experiments. The Wilson-Geneva data (table 1) are from plants rayed at Geneva grown in an "uncontrolled" greenhouse during January and February 1940.

The Nebel-Geneva data (table 2) were obtained in the same way except that for six days preceding and during the experiment the plants were grown in a climostat at 70°F., and 50 per cent humidity, being exposed twelve hours daily to the light of ten lamps. The average intensity of illumination was 0.6 lumens per square centimeter.

The Sax (Nebel-Geneva) data (table 3) were derived from cut plants rayed and prepared at Harvard by Dr. Sax, scored by Nebel at Geneva.

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The Nebel-Cold Spring Harbor data (table 4) are from cut heads rayed at Memorial Hospital in New York and counted by Giles in New Haven and by Nebel in Geneva.

The intensity data (table 5) were collected by Giles and Nebel from cut heads rayed at Memorial Hospital, at intensities ranging from 1.25 to 600 r/m; the dose at the highest intensity was administered by Mr. F. Exner, at the Presbyterian Hospital of New York. Each observer scored part of the total number of slides for each point in the experiment. The conditions of irradiation were unknown to the observers until after scoring had been completed.

Uniform score sheets were used by each observer who compiled complete records of all observations for every cell observed. Observations were made at metaphase and anaphase. Aberrations were classified as dicentrics (D.), rings, both centric and acentric (R.), rods, isodiametric fragments, minute fragments, and notches or achromatic spots. Rods related to dicentrics and rings were not used in the present compilation. Rods representing terminal deletions are extremely rare in five-day material and were thus omitted. Chromosome notches were also omitted. Tricentrics were scored as two dicentrics. Rings were recorded as centric or acentric, but only those

structures were thus classified in which the lumen of the ring was clear, solid structures falling into the category of isodiametric fragments. For the present paper, dicentrics, centric rings and acentric rings were counted in one category which will be referred to as D.R. The isodiametric fragments (I.F.), corresponding to the interstitial deletions of Sax, are solid chromatic pieces without a lumen, globular or roughly cubic in shape, ranging in diameter from that of a chromosome to that of a half-chromatid. The isodiametric fragments listed in the case of the intensity experiment, include all size classes; i.e. they comprise also minute fragments which, in the case of the data on varying dosage, are omitted. The counts are expressed as aberrations per cell or, when comparison with theory is needed, as per cent aberrations per chromosome.³

Roentgen radiation was applied as follows: In Geneva a General Electric bedside unit operating at approximately 70 kv and 3 milliamperes with a tungsten target Coolidge tube, air cooled and capa-

$$\begin{aligned} &^3 \text{ More specifically, } \% \text{ aberrations per chrom.} = \\ & \frac{100 \times \text{no. of aberrations}}{6 \times \text{no. of cells}} \end{aligned}$$

TABLE 1. Wilson-Geneva data.

Type	Radiation		Date	No. of cells	No. of heads	Results	
	Dose in r	Intensity r/m				D.R. cell	I. F. cell
						(= inflorescences)	
K ^a	55	100	1/30	182	4	.05	.04
i ^b	165	100	2/5	100	2	.18	.17
i	220	100	1/30				
			2/22	300	4	.38	.42
i	275	100	1/30-2/5	300	4	.46	.44
i	330	100	2/22	200	3	.64	.94
i	550	100	3/22	200	2	1.25	1.96
i	660	100	2/22	200	3	1.28	1.99

^a K = continuous.

^b i = intermittent irradiation.

TABLE 2. Nebel-Geneva 1940/1941 data.^a

Type	Radiation		Date	No. of cells	No. of heads	Results	
	Dose in r	Intensity r/m				D.R. cell	I. F. cell
i ^b	110	25	11/22/40	180	5	.07	.13
K ^c	110	200	11/22/40	80	4	.09	.10
i	132	40	3/13/41	111	3	.09	.15
i	440	110 av.	10/4/40	108	6	.82	1.51
			11/30/40				
i	660	200	1/4/41	38	3	1.39	2.32
K ^d	600	1600 av.	10/25/40	24	2	1.58	2.79

^a *T. reflexa* in all cases except for 132 r where *T. paludosa* was used.

^b i = intermittent irradiation.

^c K = continuous.

^d Given at Rochester, N. Y., Strong Memorial Hospital.

TABLE 3. *Sax-Harvard data.*

Radiation			Results				
Type	Dose in r	Intensity r/m	Date	No. of cells	No. of heads	D.R. cell	I. F. cell
K ^a	75	160	4/17/41	50	1	.04	.04
K	158	160	4/17/41	90	1	.16	.22
K-i ^b	320	160	4/17/41	30	1	.43	.90
K-i	472	160	4/17/41	20	1	.85	1.17

^a K = continuous.^b K-i = intermittent period of 30 seconds used to read x-ray dosimeter.TABLE 4. *Nebel-Cold Spring Harbor 1941 data.*

Radiation			Results				
Type	Dose	Intensity r/m	Date	No. of cells	No. of heads	D.R. cell	I. F. cell
						(G) ^a (N)	(G) (N)
K	110	50	7/25	300	5	.06 .12	.12 .14
K	200	100	7/25	350	7	.26 .26	.41 .35
K	400	200	7/25	150	5	1.0 .96	1.52 1.31

^a (G) as counted by Giles; (N) as counted by Nebel. Cut heads from plants grown outdoors July, 1941. 75°-90° F.TABLE 5. *Intensity effect on chromosomes.^a*

Dose	App. r/m int.	Time in min.	Date	Buds	Heads	Cells	D.R.	I.F.		
							cell	σ/\sqrt{n}	cell	σ/\sqrt{n}
300	1.25	240	7/25	7	7	280	.193	.026	.325	.028
309	2.5	120	7/17	7	6	311	.287	.019	.473	.060
300	5	60	7/10	7	6	350	.346	.039	.577	.071
300	5	60	7/25	7	7	280	.347	.023	.593	.027
309	10.2	30	7/17	8	8	343	.348	.035	.623	.072
308	20.5	15	7/3	7	7	455	.426	.049	.545	.146
308	50.1	6	7/3	5	5	350	.484	.069	.750	.061
300	50	6	7/10	8	7	351	.480	.074	.779	.066
308	154	2	7/3	3	3	124	.475	.043	.740	.167
300	150	2	7/10	7	6	375	.464	.042	.769	.064
308	616	.5	7/3	5	5	395	.540	.019	.796	.116
300	600	.5	7/10	4	4	196	.526	.059	.790	.114
309	618	.5	7/17	9	8	396	.508	.025	.777	.056
300	1200	.25	7/17	6	6	245	.541	.030	.712	.031

^a Continuous irradiation.

ble only of intermittent service—one minute on, one minute off—was used.

In New York City three sources of x-rays were used, as follows: The intensity of 600 r/m was obtained by placing the material at 15.6 cm. from the anodes of two x-ray tubes operating at about 190 kv. The filtration was 0.5 mm. Cu + 1.65 mm. of Al and H.V.L. of the radiation was found to be 0.8 mm. of Cu. The intensities of 150, 50 and 20 r/m were obtained by using only one tube of the installation at distances of 22.1, 38.2 and 60 cm. from the anode. The intensities ranging from 1.3 to 10 r/m were obtained from a constant potential machine operated at 200 kv, 4 and 8 ma current, filter of

2.11 mm. Cu at distances 50.8 and 101.6 cm. from the anode. Both installations were at Memorial Hospital and have been described elsewhere (Marinelli, 1941). The x-ray output was determined directly with a thimble chamber which had been previously calibrated against a standard ionization chamber (Quimby, et al, 1938). The material was placed on a celluloid sheet 0.25 mm. thick with light wooden supports, and it was, in all cases, sufficiently removed from objects which may have scattered or absorbed x-rays.

The third source of radiation was furnished by Mr. F. Exner, Department of Cancer Research, Columbia University. This high voltage unit oper-

ated at 475 kv equivalent constant potential 16.5 ma, .25 mm. Cu plus .45 mm. Al filters, delivering 1260 r/m at 37 cm. focal distance with absorption and scattering corrections negligible; copper H.V.L. 2.8 mm.

PART II. STATISTICAL⁴

In advance of the intensity experiments it was uncertain whether the effect of a given dose should be gauged by (a) examining several hundred cells from a single flower head or by (b) examining fewer cells from each of a number of flower heads. A working answer was reached by the following considerations.

Suppose that N cells are examined from each of n flower heads, and that X aberrations of some particular kind are found altogether. The mean number of aberrations per cell, \bar{x} , is X/Nn . Repeated samplings of the same sort would be expected to yield somewhat different means with a standard deviation (i.e. standard error of the observed mean) of the value

$$\epsilon_{\bar{x}} = \sqrt{\frac{\bar{x}}{Nn} + \frac{\sigma_H^2}{n}} \quad (1)$$

The term \bar{x}/Nn represents the variation attributable to sampling errors, that is, the variance which would be expected in repeated samples of Nn cells from essentially similar flower heads. This follows from the fact that the array of numbers of cells with various numbers of aberrations in any one experiment is, at least approximately, a Poisson distribution.

The term σ_H^2/n represents the variation attributable to differences among heads in respect to effect of a given x-ray treatment. It is the variance of \bar{x} values which would be expected in repeated very large samples each from n heads among a population of flower heads which were not alike in reaction to identical treatment.

Given that it is not practical to use more than about six flower heads per experiment, how many cells should be counted per head? The answer depends primarily upon the degree of accuracy required, i.e., on the desired value of the ratio $\epsilon_{\bar{x}}/\bar{x}$. For this ratio equal to or less than some fraction $1/r$,

$$N \geq \frac{1}{\bar{x} \left[\frac{n}{r^2} - \left(\frac{\sigma_H}{\bar{x}} \right)^2 \right]}$$

It was estimated that under the conditions of the intensity experiments (table 5) \bar{x} and σ_H/\bar{x} would be respectively greater and less than 0.2. Then for the observed D.R. values to be at least six times their standard errors, i.e., for $r = 6$,

$$N \geq \frac{1}{.2 \left[\frac{6}{6^2} - .2^2 \right]} = 40.$$

⁴ By D. R. Charles.

Accordingly forty or more cells from each of about six flower heads were examined in most of the intensity experiments.

Actually \bar{x} was between 0.35 and 0.55 in twelve of the fourteen experiments, and σ_H was found to be negligible. So the accuracy obtained was nearly twice the minimum set *a priori*: the D.R. values of table 5 are on the average 11.5 times as large as their standard errors.

In table 6 evidence is presented that inter-head variation of x-ray sensitivity is negligible. Column A is the standard deviation of D.R. means within successive experiments of table 5, calculated in the usual way from the three-to-eight head means within each experiment. Column B is the standard deviation to be expected from equation (1) if σ_H is practically zero, i.e. if the inter-head differences in D.R. means are attributable completely to sampling error. For this standard deviation among one-head samples the formula becomes $\sqrt{\bar{x}/N}$.

TABLE 6.

Int.	A	B	Int.	A	B
1.25	.069	.069	5	.060	.093
2.5	.046	.074	10.2	.099	.088
5	.096	.081	20.5	.130	.082
			50.1	.154	.083
50	.182	.098	600	.119	.102
154	.074	.107	618	.071	.102
150	.103	.086	1200	.073	.115
616	.042	.083			

Since the predicted standard deviations are higher than the observed (i.e. account for even more variation than is observed) in six of the fourteen experiments, there seems to be no reason for assuming any significant sensitivity difference between heads under the conditions of these experiments.

PART III. MATHEMATICAL INTERPRETATION⁵

Before entering into a mathematical approach to this problem, it may be well to notice that, as shown in table 7, the ratio I.F./D.R. is constant within the limits of experimental error throughout the experiments herein reported.

TABLE 7.

Experiment	Ratio I.F./D.R.
Nebel-Geneva	1.66 ± 0.15
Wilson-Geneva	1.21 ± 0.28
Nebel-Cold Spring Harbor	1.48 ± 0.26
Intensity data	1.57 ± 0.11
Sax, Harvard	1.46 ± 0.32
General average	1.48

The change in the I.F. classification already mentioned in Part I affects the I.F. to D.R. ratio by amounts which, in comparison to the average devia-

⁵ By L. D. Marinelli and B. R. Nebel.

tions, are too small to invalidate the above mentioned conclusion. It is obvious, therefore, that any mathematical expression which is adequate for the quantitative description of the D.R. phenomenon will become adequate also for the I.F. description by the mere use of a constant factor equal to 3/2; the interpretation of this factor will be left temporarily in abeyance.

If one supposes that the D.R.'s are the result of two independent events (chromosome breaks) which are reversible in some fashion (chromosome restitution), the analysis of both Swann and Del Rosario (1931) and Lea (1938) can be applied directly. Although these analyses differ only in their assumption as to the mode of recovery, their application to the data on hand will be treated separately and in reverse chronological order for reasons which will soon become obvious.

Lea's analysis is developed from the assumption that a single break fails to produce a D.R., but a second break (within definite space limits), happening within time T of the first, produces the effect. Elapse of a time greater than T between the breaks presupposes complete restitution of the first break and consequently failure to produce a D.R. In order to comply with the intensity data, however, it has been found necessary to modify slightly Lea's assumption, in that only a fraction K of these restricted double breaks are effective in producing D.R.'s.

Let N be the radiation intensity and let it be defined in terms such that Ndt is the probability of causing a break in any one chromosome in time dt ; then, according to Lea, the number of D.R.'s present per chromosome can be expressed as:

D.R./chrom. =

$$K \left\{ 1 - e^{-Nt} \left[1 + Nt + \sum_{s=2}^{\infty} \frac{(Nt)^s}{s!} \left(1 - \frac{(s-1)}{t} T \right)^s \right] \right\} \quad (1)$$

In this formula t is the irradiation time and the summation extends over integer values of S from $S = 2$ to $S = t/T + 1$. By suitable graphical analysis it is possible to reproduce the intensity data (table 5) with the following constants:

$$Nt = \frac{\text{Dose in r}}{60} = \frac{300}{60} = 5; \quad T = \frac{60}{9} = 6.7 \text{ minutes}; \\ K = 0.0786.$$

The results of the calculations are shown in the third column of table 8. It must be noted, however, that when these values of T and K are used in (1) in conjunction with the values of Nt proper to the x-ray doses used in the rest of the experiments, Lea's formula fails to produce satisfactory agreement with the experimental data.

According to Lea's formula "absolute saturation" would take place at 300 r when delivered at rates higher than 50 r/m. In other words, formula (1), in order to conform to the intensity data (table 5), sets a maximum on the number of aberrations that can be produced by any dose of x-rays, however

TABLE 8. Comparison between intensity experiment and theory. Dose = 300 r.

r/m	Exp.	% D.R./chromosome	
		Formula (1) $T = 6.7 \text{ min.}$ $K = .0786$	Formula (7) $N = 1/640$ $\times r/m$ $\lambda = 0.02$
1.25	3.22	3.2	3.12
2.5	4.78	4.5	4.62
5.0	5.77	5.8	5.95
10.0	5.8	6.9	7.12
20.0	7.1	7.6	7.44
50	8.0	7.8	7.83
150	7.8	7.8	7.99
600	8.7	7.8	8.10
1200	9.0	7.8	8.10

high. This maximum is only 7.86 per cent of the total number of chromosomes (0.46 per cell), and it is in obvious contradiction to experimental evidence.

In order to avoid this difficulty and to retain the assumption of a definite restitution time, it would be necessary to postulate that the probability N associated with any one chromosome break is not necessarily independent of either dose or time. This would require, however, a radical alteration of Lea's theory.

Swann and Del Rosario (1931) have considered the case where "two impacts of the sensitive region with an a particle are necessary to cause death of *Euglena* with a generality which allows for recovery of the cells between the two impacts."

Since it is anticipated that their theory will prove useful in explaining the existing data, it will be summarized here in some detail in terms that apply to chromosome aberrations. It will be assumed that:

(a) Chromosome breaks are produced at random by x-ray radiation at a rate proportional to the radiation intensity.

(b) The healing rate of singly broken chromosomes is numerically equal to a constant fraction λ of the total number of single breaks present.

(c) Dicentric and rings are the result of two chromosome breaks occurring within effective volumes " V_a "; the number of effective volumes V_a available is equal to the total number of chromosomes (n).

(d) I.F.'s are the result of two chromosome breaks occurring in effective volumes, V_b , which are not competing with V_a ; their total number is $3n/2$.

(e) Completed chromosome aberrations are irreversible.

It is realized that the description of the effective volumes is, from a realistic point of view, unsatisfactory, since the volumes are defined merely by their total number. A more satisfactory definition would have been possible if, for purpose of description, the scoring had been divided in isodiametric fragments and rings (involving presumably one

chromosome) on the one hand and dicentrics (involving two chromosomes) on the other.⁶

In considering the process of D.R. formation let:

t = the time of exposure.

n_0 = the number of V's in which no break has occurred or which have recovered from one.

n_1 = the number of V's in which one break has occurred and which have not yet recovered.

n_2 = the number of V's in which two breaks have occurred, namely the number of D + R.

N = the probability of causing a chromosome break within any one effective volume in unit time. It is assumed that this is proportional to the x-ray intensity and of the same magnitude for all single breaks.

The equations describing the process postulated above are:

$$\frac{dn_0}{dt} = -Nn_0 + \lambda n_1 \quad (2)$$

$$\frac{dn_1}{dt} = Nn_0 - \lambda n_1 - Nn_1 = -\frac{dn_0}{dt} - Nn_1 \quad (3)$$

$$\frac{dn_2}{dt} = Nn_1 \quad (4)$$

Summing (2), (3) and (4) term to term and integrating there follows:

$$n_0 = n - n_2 - n_1 \quad (5)$$

when the boundary conditions at $t = 0$ are taken into consideration.

By differentiating (4), multiplying (3) by N and taking into account (4) and (5) there results the

⁶ In any case it will be found useful to define the effective volumes so as to permit the use of a disposable constant coefficient; in the case of D.R. this factor need not be different from unity but in the case of I.F.'s it is assumed to be close to 3/2.

following ordinary linear equation of 2nd order in n_2

$$\frac{d^2n_2}{dt^2} + (2N + \lambda) \frac{dn_2}{dt} + N^2n_2 = N^2n$$

the general solution of which is

$$n_2 = n + A e^{-(\alpha - \beta)t} + B e^{-(\alpha + \beta)t} \quad (6)$$

where $\alpha = N + \lambda/2$ and $\beta^2 = N\lambda + \lambda^2/4$. After determination of A and B , subject to the boundary conditions $n_2 = 0$ and $dn_2/dt = 0$ at time $t = 0$, one obtains

$$n_2 = n - \frac{n}{2\beta} \left[(\alpha + \beta)e^{-(\alpha - \beta)t} - (\alpha - \beta)e^{-(\alpha + \beta)t} \right] \quad (7)$$

which is the desired relationship.

The adaptation of (7) to the intensity data was carried out by trial and error.⁷ By assuming

$$N = \frac{r/m}{640} \text{ and } \lambda = 0.02 \text{ (namely, 2 per cent of the}$$

broken chromosomes healing in one minute) good agreement was obtained with the intensity data. These values were obtained by equating expression (7) to the experimental results obtained at 5 r/m and 150 r/m which are the most reliable in the intensity experiment; the calculated values are shown in the fourth column of table 8. The data for varying doses at continuous irradiation (Nebel-Cold Spring Harbor, table 4) can be represented fairly well with the same formula and the same constants as shown in table 9.

⁷ The authors wish to thank Dr. Bertrand Goldschmidt of the Curie Laboratory, Paris, for his most generous assistance in the calculations necessary to this work.

TABLE 9.

t = 2 min. ; λ = 0.02		% D.R./Chromosome		
		Theoretical	Exp.	Theor./exp.
400 r	N = $\frac{200}{640}$	12.9	16.3	0.8
	N = $\frac{100}{640}$	3.9	4.3	0.9

TABLE 10. Comparison between formula (7) and Wilson-Geneva experiment.

$$N = \frac{r/m}{640}, \lambda = 0.02.$$

		% D.R./Chromosome		
Dosage in r	t min.	Exp.	Theor.	Theor./Exp.
660	11	21.3 (?)	26.3	1.23 (?)
550	9	20.8	20.4	.98
400	7	14.0	12.5	.89
300	5	9.2	7.85	.85
200	3	4.3	3.9	.91
100	1	1.13	1.1	.97

If $N = r \text{ m}/600$ the agreement would be better than 3 per cent at both dosages; this may mean a change of only 6.5 per cent in either radiosensitivity or in x-ray dose. Since the intensity data are the result of many more independent determinations of satisfactory accuracy, it is preferable to retain the constants as derived from them and to reserve final evaluation of N and λ by the method of least squares from different types of experiments whenever the nature of the problem will warrant it.

Formula (7) is in fair agreement also with the remaining dosage data herein presented. The magnitude of the experimental error, however, does not warrant at this moment a cumbersome extension of Swann's analysis to check the Wilson-Geneva and Nebel-Geneva dosage experiment with intermittent exposures, since in these the off-periods are too short to yield conclusive evidence on recovery. However, gross comparison between experiment and theory based on total time elapsed has been made, and the results are shown in table 10.

In search of further confirmation of Swann's formula, one finds also agreement by interpolation with that part of Sax's data (1941) which is to be considered as the most reliable, on account of explicit statements made about the calibration of the roentgen tube. Thus, in table 11 there is a comparison between his high intensity data (Sax, 1941) and theory with the same constants derived from our experiments.

In tables 12 and 13 the comparison is extended to Sax's dosage curves at 2.7 and 20 r/m. (Sax's table No. 6, 1941.)⁸

The rate of healing $\lambda = 0.02$ seems rather reasonable. Thus, according to this value, 90 per cent of the single chromosome breaks would heal within 115 minutes after their occurrence; this fact is not rigorously confirmed by direct evidence, yet it cannot be considered inconsistent with Sax's fractionation dosage experiments. It is unfortunate that the greater part of Sax's extensive data cannot be used in any crucial way to confirm or disprove Swann's theory; the numerical values available show both wide fluctuations and consistent differences with the values

TABLE 11. Comparison between formula (7) and Sax's data (1941).

$$\text{Radiation intensity} = 160 \text{ r/m}; N = \frac{r/m}{640}; \lambda = 0.02.$$

Dose in r	% D.R./Chromosome		Theor./Exp.
	Exp.	Theor.	
100	1.0	1.1	1.1
200	3.1	3.9	1.26
300	6.5	8.1	1.24
400	11.0	13.0	1.18

⁸ It is to be noted that the ratio between the theoretical values and Sax's (1941) is rather consistent for any given radiation intensity. The average ratio, (1.00 for table 12, 1.16 for table 13, and 1.19 for table 11) is seen to increase with the intensity of the radiation. This points to a small but definite difference in N of about 9 per cent which may be interpreted as above.

TABLE 12. Comparison between Sax (1941) and formula (7).

$$\text{Radiation intensity} = 2.7 \text{ r/m}; N = \frac{r/m}{640}; \lambda = 0.02.$$

Dose in r	% D.R./Chromosome		
	Exp.	Theor.	Theor./Exp.
100	1.0	.883	.883
200	3.1	2.68	.865
300	4.0	4.78	1.19
400	6.0	6.94	1.16
500	9.95	9.10	.92

TABLE 13. Comparison between Sax (1941) and formula (7).

$$\text{Radiation intensity} = 20 \text{ r/m}; N = \frac{r/m}{640}; \lambda = 0.02.$$

Dose	% D.R./Chromosome		
	Exp.	Theory	Theory/Exp.
100	1.0	1.05	1.05
200	3.1	3.73	1.20
300	6.5	7.43	1.14
400	9.95	11.7	1.18
500	13.0	16.33	1.26

presented or used in this paper. This discrepancy cannot be due to differences in scoring methods, which are essentially identical. The only difference arises from the inclusion in scoring by Sax of only centric rings rather than of all large rings. However, a correction for this factor probably would not raise his absolute values by more than four per cent.

It seems more likely that most of the discrepancy is due to inadequate radiation dosimetry; the importance of this factor has been discussed by Fabergé (1940a) and need not be emphasized here.

The recovery mechanism embodied in assumption (b) could be brought to a more direct test by means of well planned experiments with fractional x-ray doses. The general solution (6) can be used to calculate the results to be expected from two doses Nt_1 and Nt_2 of x-rays administered t_p minutes apart.

It is obvious that the effect of the first dose is adequately described by (7); during the rest period t_p , $N = 0$, hence the fundamental equations become

$$\frac{dn_0}{dt} = \lambda n_1 \quad \frac{dn_1}{dt} = -\lambda n_1 \quad \frac{dn_2}{dt} = 0$$

which give immediately:

$$[n_2]_{t_p} = [n_2]_{t_1} \text{ and } [n_1]_{t_p} = [n_1]_{t_1} e^{-\lambda t_p} \quad (8)$$

where the subscript indicates the time interval t_1 , t_p or t_2 at the end of which n_1 or n_2 are calculated. In order to calculate the effect of the second dose delivered in time t_2 , the relationships (8) are used as boundary conditions to which (6) must conform

Thus at $t_2 = 0$

$$[n_2]_{t_2} = [n_2]_{t_1};$$

hence from (6)

$$A + B = -n + [n_2]_{t_1} \quad (9)$$

Moreover from (4)

$$\left[\frac{dn_2}{dt} \right]_{t_p} = N [n_1]_{t_1} e^{-\lambda t_p}$$

hence at $t_2 = 0$, from (6)

$$(a - \beta) A + (a + \beta) B = -N [n_1]_{t_1} e^{-\lambda t_p} \quad (10)$$

Since $[n_1]_{t_1}$ can be obtained by direct differentiation of (7), namely

$$[n_1]_{t_1} = \frac{nN}{2\beta} \left[e^{-(a-\beta)t_1} - e^{-(a+\beta)t_1} \right] \quad (11)$$

the result follows immediately in the form

$$[n_2]_{t_2} = n - \frac{1}{n} \left\{ (n - [n_2]_{t_1}) (n - [n_2]_{\text{exp.}t_2}) e^{-\lambda t_p} - [n_1]_{t_1} [n_1]_{\text{exp.}t_2} \right\} \quad (12)$$

where exp. t_2 indicates that t_2 is to be used instead of t or t_1 in the exponents of formulae (7) and (11); this conventional notation should not be confused with the one adopted previously.

In the particular case of equal irradiation time intervals, namely $t_1 = t_2 = t$, (12) reduces to

$$[n_2]_{t_2} = n - \frac{1}{n} \left\{ (n - [n_2]_{\text{exp.}t})^2 - e^{-\lambda t_p} [n_1]_{\text{exp.}t}^2 \right\} \quad (13)$$

It is evident that for $t_p = \infty$ the baseline as defined by Sax (1939, 1941) is

$$[n_2]_{t_2; t_p = \infty} = n - \frac{1}{n} \left[n - (n_2)_{\text{exp.}t} \right]^2 \quad (14)$$

and that the effect of the uninterrupted dose ($t_p = 0$) for time $2t$ is:

$$[n_2]_{2t} = n - \frac{1}{n} \left\{ (n - [n_2]_{\text{exp.}t})^2 - [n_1]_{\text{exp.}t}^2 \right\} \quad (15)$$

By subtracting (14) from (15) and solving for $[n_1]_{t_1}$, the number of single breaks existing at the end of the first irradiation, one obtains.

$$[n_1]_t = \sqrt{n \left\{ [n_2]_{2t} - [n_2]_{t_2; t_p = \infty} \right\}} \quad (16)$$

This expression indicates another convenient, though indirect, experimental check on the theory; namely although $[n_1]_t$ cannot be determined directly by observation (because of the recovery taking place between radiation of the material and observation of results), it can be calculated from experimental

values by means of (16) and compared with the theoretical predictions of (11).⁹

The recovery constant λ is also capable of direct experimental determination. In fact, when (16) and (14) are substituted in (13) one obtains

$$-\lambda t_p = \log \frac{[n_2]_{t_2} - [n_2]_{t_2; t_p = \infty}}{[n_2]_{2t} - [n_2]_{t_2; t_p = \infty}} \quad (17)$$

which shows that the semi-logarithmic plot of the fraction in the right hand term against the rest period t_p should be linear with a slope (independent of both time and dosage used in the irradiation) equal to the recovery constant λ . The experimental determinations of (16) and (17) are apt to be of little value unless the denominator of the right hand of (17) is sufficiently large. This requires high radiation intensities with short exposure time, fairly high dosage, and large numbers of chromosomes. Thus, if the radiation is administered in two doses of 200 r of one minute each, the number of per cent D.R. per chromosome will be 12.9 when $t_p = 0$ and 7.64 for $t_p = \infty$. The difference 5.26 represents a 40 per cent change in the total number of D.R.'s. Therefore, to obtain an accurate value of λ , the D.R. values for the intervening t_p 's must be known to within a few per cent.

The usefulness of the fractional method, however, will increase with higher radiation doses and with detailed aberration scoring. Its careful application will help to clarify the limits within which the simplified assumptions herein made are applicable, as well as to determine the possibility of a variation of the rate of recovery λ with dose, as suggested recently by Newcombe (1942b). It is anticipated that, apart from the latter phenomenon, provision may have to be made in Swann's theory to account for partial obliteration of two-break aberrations at higher doses, owing to the frequent occurrence of more than two breaks within the effective chromosomal volumes. It remains to be seen, also, whether aberrations are subject to the same probability of break (N not constant on account of chemical changes in chromosomal or cytoplasmic material), and whether reclassification of morphological processes is necessary to explain the aberration data that Newcombe and Fabergé have obtained at much higher x-ray doses.

GENERAL CONCLUSIONS.—The power law concept used so often in the literature dealing with the quantitative aspects of x-ray chromosome aberrations has not been helpful in interpreting the data on D.R.'s and I.F.'s, beyond the point of suggesting the possibility of their being caused by double chromosome breaks. Swann and Del Rosario's theory of recovery accounts for most anomalies in the data herein presented or reviewed. Rather simple calculations based on their formula will show, for instance, that the differences between experiments at

⁹ For all practical purposes $t_p = \infty$ is reached after about three hours.

constant intensity and those at constant time should exist at low but not at high x-ray intensities; they will show, also, that the time intensity factor at low doses, while not negligible, is nevertheless relatively less important than at higher doses.

Swann and Del Rosario's theory is a description of a morphological event of microscopic dimensions and requires no assumption as to the primary effect of x-rays on chromosomes or cytoplasm beyond the requirement that the rate of production of breaks be proportional to the x-ray intensity.

The evidence presented in this paper favors the type of recovery postulated by Swann and Del Rosario, namely that the probability of a single chromosome break healing in the next unit time is a constant λ independent of x-ray dose and of irradiation time. The type of recovery assumed by Lea, namely that restitution of chromosome breaks occurs within a fixed time, fails to conform with experiment.

The assumption of Swann's theory do not conform with Darlington and Upcott's (1940) interpretation of Sax's (1939, 1940) intensity experiments, namely with the hypothesis that during the first hour after breakage the proportion of breaks which are lost by restitution depends on the competition for reunion among broken ends at the time of splitting. This mechanism presupposes formation of D.R.'s and I.F.'s after cessation of irradiation (contrary to equation (4) which gives $dn_2/dt = 0$ for $N = 0$) and a dependence of the recovery probability λ of a fresh break on the number n_1 of the single breaks existing in the chromosomal material (contrary to Swann's assumption $\lambda = \text{constant}$). The hypothesis of Sax (1939), that in the course of time the first break may heal before a second break occurs in an adjacent chromosome, is consistent with the fundamental equations.

The foregoing mathematical interpretation at present is to be considered valid only for low doses of radiation, since it neglects the possible effects of radiation on the immediate environment of the chromosomes and ignores the possibility of loss of chromosome aberrations scored through overlap of events within the same chromosomal pieces.

SUMMARY

The quantitative data herein presented on the action of x-rays on chromosomes represent the results of a study carried out with standard material and equipment. Scoring of the aberrations produced has been obtained by independent observers, according to conventional terms and definitions. Experimental errors and the size of sample necessary for adequate precision have been determined from statistical theory.

The data herein presented, as well as the most recent data of Sax (1941), have been interpreted satisfactorily in the light of a theory presented by Swann and Del Rosario in their study of the effect of radioactive radiation upon *Euglena*. Moreover, an extension of this theory is presented to predict results to be expected from fractional x-ray doses, and suggestions are advanced as to the experimental prerequisites of these investigations.

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THE SUPPLY OF WATER TO TRANSPIRING LEAVES ¹

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THE MAINTENANCE of a favorable water balance in the leaves of transpiring plants demands that loss of water from the leaves shall not, except for very short periods, exceed the supply of water to them. The available balance of water in the leaves which can be expended in transpiration without the onset of wilting is surprisingly low in mesophytes (Knight, 1922). If, therefore, wilting and its consequences are to be avoided, a regular flow of water to the leaves is essential. With succulent plants, possessing special water storage tissue, the position is different, while with many non-succulent xerophytes a considerable reduction in the water content of the leaves may occur without damage to the leaf resulting. During its passage from the soil to the leaves, water first enters the root hair and then passes through the cortex, endodermis and pericycle to the xylem, in which tissue it is transported to the foliage leaves. At any stage in its transport the water may encounter resistances to its passage, the resistances varying in magnitude from plant to plant and probably from time to time within the same plant. It is the purpose of this paper to discuss the resistances offered to water transport by both root and stem and the mechanism by which these resistances are overcome.

STEM RESISTANCE.—Various determinations of the ability of stems to transmit water have been made (Dixon, 1914; Farmer, 1919; Warne, 1937), and it appears that the resistance offered to the movement of water in the vascular system of the stem is not great. Dixon with *Taxus baccata* showed that, to obtain a velocity of upward water transport in the stem sufficient to cover the transpiration requirements, needed a pressure equivalent to a head of water twice the length of the stem. If Dixon's conclusions are generally applicable, then in the tallest trees a pressure of approximately 23 atmospheres is necessary to raise water to the level of the highest leaves. The osmotic pressure of the leaves of trees is often sufficient to supply such a pull to the water column in the tracheae, while expressed plant saps are capable of transmitting a pull of this magnitude owing to their cohesive properties (Dixon, 1914). In shrubs and herbaceous plants the necessary pressure is much smaller. In a small shrub 200 cm. in height a pressure of only about 0.5 atmosphere is needed. Farmer (1919), however, showed that the conductivity of the woods of different species varied considerably. The specific conductivity, e.g., the amount of water transmitted through a 15 cm. length of stem in fifteen minutes under a pressure of 30 cm. mercury divided by the area of cross section of the conducting tract, was higher for deciduous than for evergreen trees and shrubs. The values found varied from 1.0 for *Ruscus aculeatus* to 95 for the

Osier (*Salix viminalis*). These data, however, are not easy to interpret. When considering the resistance offered by the stem to the flow of water to the leaves, it is desirable to take into account the area of transpiring foliage which has to be supplied. Specific conductivities alone may be misleading. A low specific conductivity may be more than counterbalanced by a large amount of conducting tissue or by a reduction in the transpiring surface. It seemed desirable, therefore, to determine conductivities of the shoots of a number of species and to express these conductivities on the basis of area of foliage to be supplied. This has been done, and different species are found to give values which vary widely.

Experimental.—Determinations have been made of the conductivities of the stem of a number of species and measurements of leaf area made simultaneously. The conductivity measurements have been made on two-year old shoots, and the total area of foliage carried by the shoot above the piece of stem used for the conductivity measurement has been determined. With one species, *Buxus sempervirens*, the amount of water transmitted through a two-year old stem was so small that older stems (four to five years) were used. All the material came from the University Experimental Grounds and so had been grown under uniform conditions of soil and climate. The apparatus and method used have been described in a previous paper (Warne, 1937). The data collected are assembled in table 1.

In column two are given data for absolute conductivities, or the amount of water in grams transmitted through a 15 cm. length of stem under a pressure of 30 cm. of mercury in fifteen minutes, while in column 3 the conductivities are expressed as grams of water per 100 square cm. of foliage carried by the

TABLE 1.

Species	Mean values \pm standard errors	
	Absolute conductivity. gms. water	Conductivity per 100 cm ² foliage
<i>Rhododendron ponticum</i>	0.50	0.330 \pm 0.0388
<i>Ilex aquifolium</i>	0.61	0.323 \pm 0.0351
<i>Prunus Lauro-cerasus</i>	0.97	0.289 \pm 0.0239
<i>Ruscus aculeatus</i>	0.055	0.043 \pm 0.0029
<i>Fraxinus excelsior</i>	11.56	0.793 \pm 0.0740
<i>Olearia hastii</i>	0.56	0.250 \pm 0.0320
<i>Berberis vulgaris</i>	0.234	0.148 \pm 0.0308
<i>Hedera Helix</i>	1.15	0.280 \pm 0.0377
<i>Azalea mollis</i>	1.63	0.578 \pm 0.0512
<i>Philadelphus</i> sp.	2.88	0.425 \pm 0.0112
<i>Pyrus malus</i> (Crab)	1.75	0.556 \pm 0.0356
<i>Skimmia japonica</i>	0.60	0.079 \pm 0.0157
<i>Syringa vulgaris</i>	2.37	0.362 \pm 0.0361
<i>Buxus sempervirens</i>	0.21	0.084 \pm 0.0079
<i>Castanea vulgaris</i>	4.37	0.895 \pm 0.0931
<i>Acer pseudo-platanus</i>	4.52	0.725 \pm 0.0610

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shoot. Usually about twenty determinations were made for each species. All the determinations were made during late summer when extension growth for the year had ceased but before leaf fall occurred.

Farmer (1919) found a lower specific conductivity in evergreens than in deciduous trees and shrubs. The data presented in the table also show a lower conductivity for the evergreens, although the conductivity here is expressed on the basis of area of foliage carried by the shoot.

Discussion.—The interpretation of the data presented is not easy. There is no doubt that the resistance offered to the flow of water to the transpiring leaves by the stems varies considerably from species to species, but whether the resistance is in fact a serious obstacle to the flow of water to the leaves is open to question. The data presented refer to the conductivity of 15 cm. pieces of stem, whereas in the intact plant not only is the conductivity of a unit length of stem important but it is necessary in considering stem resistance to take cognizance of the whole distance over which the water has to be transported. Of the species for which data are presented, the simplest case from this point of view is that of *Ruscus aculeatus*. The shoots of *Ruscus aculeatus* used in this work were all cut near ground level and the length of the shoot was from 50 to 60 cm. In *Ruscus* the diameter of the stem and probably also its conductivity does not diminish sensibly until the level of the lowest transpiring cladodes is reached, and above this point the diameter and conductivity probably decrease more or less in proportion to the decrease in the transpiring surface above any particular point on the stem. Hence, no very serious error is introduced if for a moment we look upon the stem as though it were of uniform diameter with all the transpiring cladodes aggregated at the apex. A pressure of 30 cm. of mercury is sufficient to cause water to be transmitted through a 15 cm. piece of stem at the rate of 0.055 gm. in fifteen minutes or 0.220 gm. per hour, equivalent to 0.172 gm. water per hour per 100 sq. cm. of cladode carried by the shoot. To transmit this amount of water through 60 cm. of stem requires a pressure of the order of 120 cm. of mercury (1.6 atmos. approximately). This supply of water barely equals the highest rate of transpiration recorded here for *Ruscus*. With cut shoots of *Ruscus* standing with their basal ends in water in a laboratory where the temperature reached 28°C. and in bright sunlight the mean transpiration rate recorded over a period of four hours was 0.208 gm. per hour per 100 sq. cm. of cladode. To cause a transmission of water at this rate a pressure of approximately 1.84 atmospheres is needed. This is greatly in excess of Dixon's estimate of the necessary pressure required for *Taxus baccata*. *Ruscus aculeatus*, however, is an extreme case. Not only has it the lowest specific conductivity of the species tested by Farmer (1919), but it has also the lowest conductivity per 100 sq. cm. of transpiring surface. On the other hand the distance over which the water has to be transported is relatively small in *Ruscus*.

Nevertheless, even in the extreme case of *Ruscus aculeatus*, the pressure needed to overcome the resistance of the stem to water transmission is negligible compared either with the pull that transpiring leaves can exert or to the tension which expressed plant sap is capable of transmitting. Stem resistance is likely to be of no greater importance in the other species, although in the other shrubs and trees, which are larger than *Ruscus*, water has to be transported over a greater distance but through wood with a lower resistance. A similar conclusion was reached as a result of an experiment of a different nature. Determinations were made of the transpiration rate from shoots of *Ruscus aculeatus* in which the length of the stem was varied so that the resistance offered by the stem to the flow of water differed in the different shoots used. The procedure adopted was to select shoots of *Ruscus* with at least 30 cm. of bare stem below the level of the lowest cladode so that about 50 per cent of the shoot consisted of bare stem.

Determinations were made of the transpiration rates of these shoots and also of shoots from which most of the lower bare stem had been removed, so that in the latter shoots the stem resistance had been reduced to a level considerably below its original value. The determinations were made by standing the shoots with their basal ends in water covered with a film of oil and weighing them at intervals of a few hours. Experiments of shorter duration were not considered desirable owing to the possibility that transpiration might take place over short periods, largely at the expense of the water present in the shoot. That this did not occur in the experiments is shown by the fact that the transpiration rates did not sensibly diminish over a period of four days, and during this period loss of water by transpiration greatly exceeded the total original water content of the cladodes. The results obtained were of an entirely negative character, and it does not seem necessary, therefore, to present them in full. Both under conditions favoring high transpiration (temperature 20°C. and bright sunlight) and low transpiration (dull and humid) there was no difference in the transpiration rates for the two sets of shoots. Removal of half of the stem with a consequent reduction in stem resistance did not effect any increase in the transpiration rate. This was so for shoots standing in water and, therefore, with a good water supply. Similar results were obtained for *Rhododendron ponticum*. It appears likely, therefore, that in the species tested here and probably in others also, the resistance offered by the stem to water transport does not constitute any serious obstacle to the flow of water to the transpiring foliage, at least when the supply of water to the shoot is optimal. These results are of special interest in view of the fact that Kramer (1938) found that removal of the root system did with some plants increase the transpiration rate and suggests that stem resistance may be of less importance than root resistance. When the water supply is poor, the additional resistance

to water flow offered by a stem of low conductivity may be of greater importance. The relation between low stem conductivity and the possession of evergreen xeromorphic foliage may well be more than coincidence. Although experiments have failed to show that stem resistance offers any serious obstacle to the flow of water to the transpiring organs, we cannot conclude that the stem of the evergreen of low conductivity could under all conditions transmit a sufficient amount of water to cover the requirements of a mesophytic foliage. In spite of the suggested unimportance of stem resistance, it is well known that the leaves of plants often do wilt when subjected to conditions favoring high transpiration even when the supply of water to the roots is good. It is likely, therefore, that other resistances to the flow of water to the foliage, more important than stem resistance, are operative.

ROOT RESISTANCE.—When a well watered, actively growing plant is decapitated just above the soil level, liquid frequently exudes from the surface of the cut stump. It is generally believed that the liquid is exuded from the cut ends of the xylem vessels and tracheids (Stiles, 1936; Kramer, 1940). That this is the correct interpretation of the phenomenon has been disputed (James and Baker, 1933). Not merely is liquid exuded, but considerable pressure (root pressure) may be set up. It is commonly assumed (Thomas, 1935) that in the intact plant the root pressure is such that water may be forced some distance up the stem so that the root system may be a positive aid to the upward transport of water in the plant. The data which will be presented here support the view that, at least under conditions favoring fairly high transpiration rates, it is more correct to look on the root system as constituting a resistance to the flow of water from the soil to the transpiring leaves.

Preliminary experiments.—When measurements are made of the rate at which liquid is exuded from the stump of a decapitated plant, it is found that this usually does not equal the rate at which water is lost by an intact plant in transpiration under normal conditions. A few data will illustrate this point. The transpiration determinations were all made by weighing potted plants at intervals, the pot being encased in a waterproof covering, under ordinary conditions in the laboratory. After a few hours the plants were decapitated, and the rate of exudation from the stump was determined.

In no case here is the exudation as great as the transpiration, while not infrequently no exudation occurs (Kramer, 1941). The root system alone,

therefore, does not exude sufficient liquid to cover the transpiration requirements of the plant. In the intact plant sufficient water is transmitted through the root system to cover the transpiration requirements. In the intact plant, therefore, water is supplied to the shoot at a rate which is in excess of the rate at which the root system unaided could supply it, as shown by the rate at which liquid exudes from the stump of a decapitated plant. Under these conditions the root system constitutes a resistance to the flow of water, a fact already pointed out by Kramer (1938).

Before going on to present estimates of the magnitude of the root resistance, an attempt will be made to show that the liquid which exudes from a cut stump is, in fact, derived from the cut ends of the xylem elements. Determinations have been made of the rate of exudation from intact stumps of *Bryophyllum pinnatum* and also from stumps, the outer tissues of which have been peeled back from the cut surface and the cambium carefully scraped off the exposed xylem surfaces. It was found that exudation took place at the same rate from both peeled and unpeeled stumps, provided that in the former all exposed surfaces of xylem, etc., were covered with a thin film of grease.

TABLE 3. Showing rate of exudation from intact and peeled stumps of *Bryophyllum pinnatum* in cc. per hour over a twenty-four-hour period.

	Intact	Peeled
	0.061	0.030
	0.033	0.047
	0.056	0.067
	0.060	0.053
	0.033	0.042
	0.057	0.050
Mean	0.050	0.048

Exudation from both peeled and intact stumps was still proceeding actively seven days after the experiment was set up. There is no doubt that here the exudate is derived from the central xylem core, though whether from the living or non-living elements of the xylem it is not possible to say. It is very doubtful, however, whether the few living cells of the xylem could exude liquid at the rates shown in table 2. This conclusion agrees with that of Kramer (1940) and lends no support to the views of James and Baker (1933). The assumption that the exudate is, in fact, derived from the cut ends of the vessels and tracheids of the xylem is an essential point in considering the data which will be presented later.

Experiments on root resistance.—Attempts have been made to get an estimate of the magnitude of root resistance in a number of species of plants and to assess its importance so far as the general water relations of the plant are concerned. The method adopted has been as follows. The rate of transpiration of an intact potted plant has been determined in the manner described earlier in this paper. The

TABLE 2.

Species	Transpiration gm./hour	Exudation gm./hour
<i>Strobilanthes</i> sp.	0.5 gm.	0.14 gm.
<i>Pelargonium</i> sp.	1.3 gm.	0.10 gm.
<i>Bryophyllum pinnatum</i>	0.10 gm.	0.02 gm.
<i>Impatiens noli-metangere</i>	0.28 gm.	0.19 gm.

transpiration rates of all the plants used could not be determined on the same day but all determinations were carried out as far as possible under uniform conditions. During the transpiration determinations the temperature was always within the limits 21°C. to 22.8°C. with relative humidity between 70 and 75 per cent, and the soil of the pots was well watered. All determinations were made in a greenhouse during periods of sunlight of moderate intensity. To avoid, as far as possible, errors due to transpiration taking place mainly at the expense of the water content of the leaf, the determinations were made over a four-hour period. In no experiment did any wilting of the leaf take place, and it has been assumed, therefore, that the observed transpiration rates represent the rate at which, in the intact plant, water was being supplied to the transpiring shoot. After completion of the transpiration determination, the plants were decapitated near the soil level and again well watered. A graduated pipette was then fitted to the stump, and recently boiled and cooled distilled water was introduced into the pipette. To avoid drying of the cut surface decapitation was done under a stream of water. A negative pressure was applied to the upper end of the pipette by means of a water pump, and the rate at which the level of the liquid in the pipette rose was determined. A negative pressure of 70 cm. of mercury was used. With an efficient water pump connected direct to the main this pressure could be maintained with no sensible fluctuations for several hours. Certain precautions had to be observed. All joints needed to be wired. As soon as the negative pressure was applied, an immediate small rise of the level of the liquid in the pipette occurred. This was due to contraction of the rubber tube used for making the joints, and the level of the liquid fell to its original value when the pressure was released. At the commencement of an experiment, air bubbles were often pulled out of the stump for a few seconds, and no accurate reading of the level of the liquid in the pipette could be made until this bubbling ceased, and this usually occurred after a few seconds had elapsed. Under these conditions liquid was sucked from the root system and continued to be delivered for some hours as shown by the data below for a plant of *Bryophyllum pinnatum*.

The rate of exudation falls off with time. This is almost certainly due to blocking of the cut surface, as the exudation rate is restored to its original value

TABLE 4. Exudation from a stump of *Bryophyllum pinnatum* under a negative pressure of 70 cm. of mercury.

Time	Amt. of exudation in cc.
11.00 a.m.	...
11.24	0.10
11.40	0.18
1.45	0.60
2.45	0.80

if a fresh surface of the stump is cut. That the exudation is derived from the xylem is shown by the fact that exudation takes place at the same rate from peeled and intact stumps. The rate of exudation is affected by the magnitude of the pressure employed. At very low pressures the rate of exudation was so slow that accurate readings could not readily be made, but table 5 shows the rate of exudation for two pressures, namely 35 cm. and 70 cm. of mercury, from a stump of *Bryophyllum pinnatum*, a fresh surface being cut before the second determination. Pressures greater than 70 cm. of mercury could not be employed.

TABLE 5. Rate of exudation from a stump of *Bryophyllum pinnatum*.

Pressure cm. of mercury	Rate of exudation cc./hour
35	0.32
70	0.63

The rate of exudation is seen to increase as the pressure used increases.

The main results obtained are assembled in table 6. Altogether data for thirty-eight plants are given representing eighteen species. Column 2 shows the rate of transpiration in grams per hour for the intact plant and column 3 the rate of exudation from the stump of the same plant under a negative pressure of 70 cm. of mercury. The exudation rates are given in grams per hour. Since the exudation rate from a stump falls off in time, these determinations were carried out over a period shorter than one hour (usually fifteen minutes), and from this figure the rate per hour was calculated. By this means the effect of the falling off, which is believed to be due to blocking of the cut surface, is minimized. Column 4 shows the rate of exudation expressed as a percentage of the transpiration rate. When more than one plant of any species was used, the mean value for that species is incorporated in the table.

The absolute values for both the transpiration and exudation rates have, of course, little significance, since the values obtained will depend largely on the size of the plants. Actually all the plants used were fairly uniform in height (25 to 30 cm.) but they differed widely in the areas of transpiring foliage which they carried. Nevertheless fairly satisfactory agreement is shown between duplicates of the same species, especially when the exudation is expressed as a percentage of transpiration. Considerable variation is shown by the plants of *Bryophyllum pinnatum* but duplicates of other species agree well. The values found vary widely from species to species, from 7 per cent for *Hakea* and *Erica* to 77 per cent for *Pelargonium sanicifolium*.

The opportunity was taken to compare root and stem resistance for a few plants. To do this the rate at which water was transmitted through a 15 cm. length of stem was determined for a negative pressure of 70 cm. of mercury in the way previously

TABLE 6.

Experiment number	Species	Transpiration rate gm/hour	Exudation rate gm/hour	Exudation as percentage of transpiration
1	<i>Bryophyllum pinnatum</i>	0.22	0.16	73
2	<i>Bryophyllum pinnatum</i>	0.62	0.22	35
3	<i>Bryophyllum pinnatum</i>	0.40	0.24	60
4	<i>Bryophyllum pinnatum</i>	0.27	0.20	74
5	<i>Bryophyllum pinnatum</i>	0.16	0.20	125
14	<i>Bryophyllum pinnatum</i>	0.76	0.40	54
15	<i>Bryophyllum pinnatum</i>	1.40	0.63	45
	Mean	0.55	0.29	67
6	<i>Bryophyllum diagremontianum</i>	0.27	0.14	52
16	<i>Bryophyllum diagremontianum</i>	0.36	0.16	44
	Mean	0.32	0.15	48
7	<i>Pelargonium grandiflorum</i>	1.27	0.44	34
8	<i>Pelargonium grandiflorum</i>	0.98	0.40	41
	Mean	1.12	0.42	38
10	<i>Pelargonium cuculatum</i>	1.11	0.48	43
11	<i>Pelargonium cuculatum</i>	0.71	0.36	51
	Mean	0.91	0.42	47
12	<i>Pelargonium ternatum</i>	0.88	0.60	68
9	<i>Pelargonium vitifolium</i>	0.62	0.32	52
34	<i>Pelargonium sanicifolium</i>	3.66	2.80	77
13	<i>Crassula arborescens</i>	0.55	0.28	52
18	<i>Quillaya Braziliensis</i>	1.42	0.15	10
19	<i>Quillaya Braziliensis</i>	2.30	0.41	18
	Mean	1.86	0.28	14
20	<i>Hakea saligna</i>	3.74	0.29	8
21	<i>Hakea saligna</i>	4.04	0.26	6
22	<i>Hakea saligna</i>	3.34	0.22	7
	Mean	3.71	0.26	7
28	<i>Hydrangea hortensis</i>	2.13	0.90	42
29	<i>Hydrangea hortensis</i>	1.78	0.80	45
30	<i>Hydrangea hortensis</i>	2.38	0.80	34
31	<i>Hydrangea hortensis</i>	1.70	0.70	41
32	<i>Hydrangea hortensis</i>	2.50	0.75	35
	Mean	2.10	0.79	39
38	<i>Hydrangea hortensis</i> (rooted hardwood cuttings)	1.40	0.64	46
39	<i>Hydrangea hortensis</i> (rooted hardwood cuttings)	1.75	0.52	30
40	<i>Hydrangea hortensis</i> (rooted hardwood cuttings)	1.00	0.32	32
	Mean	1.35	0.49	36
43	<i>Erica</i> ; President Felix Faure	2.85	0.20	7
44	<i>Acacia juncifolia</i>	1.15	0.10	9
45	<i>Acacia juncifolia</i>	1.60	0.12	7.5
	Mean	1.40	0.11	8
36	<i>Gomphocarpus macroglossus</i>	2.72	0.36	13
37	Tomato (<i>Solanum esculentum</i>)	2.00	0.50	25
35	<i>Manihot</i> sp.	1.05	0.24	23
33	<i>Solanum pseudocapsicum</i>	3.38	0.84	25
50	<i>Psidium</i> sp.	4.90	0.85	18

described, and this was compared with the exudation rate from the stump of the same plant when a negative pressure of 70 cm. was applied. The data so obtained are incorporated in table 7.

The data emphasize the unimportance of stem resistance in affecting the flow of water to the transpiring leaves and confirm the suggestion previously made in this paper and by Kramer (1938) that the

TABLE 7. Root and stem resistance of five species.

Species	Exudation rate gm/hour	Conduction of 15 cm. piece of stem gm/hour
<i>Bryophyllum pinnatum</i>	0.16	9.0
<i>Bryophyllum pinnatum</i>	0.22	6.0
<i>Bryophyllum pinnatum</i>	0.24	6.6
<i>Bryophyllum pinnatum</i>	0.20	7.2
<i>Bryophyllum pinnatum</i>	0.22	8.0
<i>Bryophyllum diageomontianum</i> ..	0.14	15.0
<i>Pelargonium ternatum</i>	0.88	37.5
Tomato (<i>Solanum esculentum</i>) ..	0.50	57.0
<i>Manihot</i> sp.	0.24	60.0

resistance offered by the stem to the upward flow of water is of relatively little importance.

Reference to the main table shows that only with a single plant of those tested did the rate at which liquid could be sucked from the root system equal the rate at which the intact plant lost water by transpiration. As pointed out previously the actual values for the exudation rate have little significance. The rate at which liquid is delivered from the stump is almost certainly affected, not only by the structure of the root but by the distance of the absorbing region of the root from the cut surface of the stump and by the extent of the absorbing surface. Since the plants used were all growing in pots, differences due to these factors were probably not so great as they would have been with plants with an unrestricted root range. The differences in transpiration rate observed reflect, not only any inherent differences in the rate of transpiration, but also differences in the area of transpiring foliage carried by the plants. The most interesting statistics are those of column 4 showing the exudation as a percentage of transpiration. Here there is a large variation between species. The succulents give fairly high values, especially the two species of *Bryophyllum*. The values for *Hydrangea hortensis* and the various species of *Pelargonium*, too, are fairly high, two species of *Pelargonium* giving values higher than the *Bryophyllum*. The lowest values are all given by the three xeromorphic plants, *Erica*, *Hakea* and *Acacia*. *Gomphocarpus* and *Quillaya* give somewhat higher values, while the other species (represented only by single observations) occupy an intermediate position. In general the succulents and mesophytes tend to give high values and the xerophytes low values. The values given, of course, refer only to the conditions of this particular experiment.

Discussion.—In the intact plants when transpiration is not taking place at the expense of the water content of the foliage, the root system is supplying water at the same rate that water is being lost from the shoots by transpiration. When a negative pressure of 70 cm. of mercury is applied to the stump of a decapitated plant the contents of the tracheae and of the stump will be subjected to a tension equivalent to 70 cm. of mercury. As the experiments which

have been described show, such a tension is not sufficient to cause water to be delivered from the root at a rate sufficient to cover the transpiration requirements of the plant. To ensure a supply of water sufficient for transpiration needs, the contents of the tracheae must be under a tension considerably in excess of 70 cm. of mercury. This suggestion makes it unnecessary to suppose that the passage of water from living cells of root to the tracheae is due to the osmotic pressure of the tracheal contents. It is not osmotic pressure but the suction force of the tracheal contents which is important. With a cut stump such as is usually employed for root pressure experiments, exudation is probably due mainly to the osmotically active substances present in solution in the tracheal contents. In the intact plant when transpiration is active the suction force may be due, in part, to the presence in the tracheae of a solution which exerts a considerable osmotic pressure, but it is probably due to a greater extent to the tension of the tracheal contents (Kramer, 1932). A conducting system which is surrounded by living cells with semi-permeable protoplasts will exert a suction force if its contents are in a state of tension, even if the liquid in the system does not contain osmotically active substances in solution. The existence of such a tension, therefore, will be sufficient in itself to cause a flow of water from the living cells of the root to the tracheae as long as the tension is greater than the suction force exerted by the surrounding living cells.

Evidence has been presented to show that the tension required to overcome the resistance of the root to water transmission varies considerably from species to species. While such tensions may be well below the tensions which expressed plant sap can transmit, there is the possibility that they at least approach the maximum pull which the transpiring leaves can exert, at the same time maintaining a condition of turgidity. If we accept the view of Atkins (1916) and look on the whole of the living cells of the root as constituting a semi-permeable membrane separating the soil solution from the xylem contents, then the suction force of the tracheal contents causes an inward flow of water from soil to xylem against the resistance offered by the living cells to water transport. The suction force necessary is, it is suggested, supplied largely by the tension of the tracheal contents. The resistance to water movement in the leaves is known to consist mainly of the resistance offered by the mesophyll cells (Mer, 1940). Even the finer veins offer little resistance to water movement. It is likely that, in the same way, the important resistance offered to water movement by the roots is that of the living root cells.

THE TENSIONS OF THE CONTENTS OF THE TRACHEAE AND THE OSMOTIC PRESSURE OF THE CELL SAP OF THE LEAVES.—It has been shown that the rate at which a decapitated stump of a plant exudes liquid, when subjected to a negative pressure of 70 cm. of mercury, rarely equals the rate of transpiration from the intact plant when conditions favor a relatively

high transpiration rate. It was concluded, therefore, that under these conditions the contents of the tracheae must be under a considerable tension. If we assume that the rate of exudation is proportional to the pressure employed, we can get an estimate of the tension which must have existed in the tracheae of the intact plant. This has been done and the values so obtained checked against direct observations on the relative tensions existing in the tracheae of the plants used. The values found by the two methods show a close correspondence.

Experimental.—Estimates of the tensions of the tracheal contents of intact plants were obtained by assuming that the rate of exudation from the stump was proportional to the magnitude of the negative pressure applied to the stump and from a knowledge that, over an extended period in the absence of wilting, transpiration is equal to the supply of water from the root.

This method of estimating the tensions may involve considerable errors, so that too much stress will not be laid on the absolute values given. The errors will apply to all the estimates made, so that any classification of the plants used based on differences between the estimated tensions in the tracheae is likely to be accurate.

Direct observations of the relative tensions in the tracheae were made using a method previously employed by Baker and James (1933). The method consists of fixing a small plasticine cup to the stem of the plant, filling the cup with dye solution (1 per cent acid fuchsin), rapidly piercing with a sharp knife the stem underneath the surface of the dye solution and determining the extent to which the dye immediately penetrates both upwards and downwards in the xylem. In these experiments the plasticine cups were fixed near soil level and so only the extent of the upward penetration could be determined. The extent of the dye penetration will give a

measure of the tension of the tracheal contents provided material of uniform size is used. The plants used in these experiments were chosen for their uniformity in height, all being about 30 cm. high. The determinations were carried out in a greenhouse with air temperature 23°C. and relative humidity 65 per cent, with sunlight of moderate intensity, and the plants were all well watered so that the conditions were similar to those which prevailed when the transpiration determinations on which the calculated tension values are based were made.

Determinations of the osmotic pressure of the sap expressed from the leaves of some of the plants used were made. The osmotic pressures were calculated from the freezing point of the sap expressed from leaves which had been killed by immersion in liquid air.

The results obtained are assembled in table 8. In column 2 are given the values for the calculated tensions. The values given are in atmospheres. Only mean values are given, the number of values on which the mean is based being shown in parentheses. Column 3 shows the penetration of dye in the xylem in cm. Mean values only are given, the number of observations on which the mean is based being shown as before. In the fourth column are the values for the osmotic pressure of the sap expressed from the leaves. For the sake of convenience the species here are listed in the order of magnitude of the calculated tensions.

Reference to table 8 shows that the two methods employed for obtaining estimates of the tensions of the tracheal contents have given results which agree well. In the succulents and mesophytes the tension tends to be low and in the more xerophytic species to be high. This of course refers only to the plants under the conditions of these experiments. It is easy to show that the tensions undergo marked variations with changes in external conditions. This is shown

TABLE 8.

Species	Calculated tension atmospheres	Dye penetration in cm.	Osmotic pressure of leaf sap in atmospheres
<i>Pelargonium sanicifolium</i>	1.21(1)	4.5(1)	9.0
<i>Pelargonium ternatum</i>	1.35(1)	4.0(1)	...
<i>Bryophyllum pinnatum</i>	1.59(7)	3.8(6)	8.3
<i>Pelargonium vitifolium</i>	1.79(1)	4.0(2)	8.6
<i>Crassula arborescens</i>	1.81(1)	8.6
<i>Bryophyllum diagremontianum</i>	1.93(2)	7.6
<i>Pelargonium cuculatum</i>	1.97(2)	3.5(1)	...
<i>Pelargonium grandiflorum</i>	2.46(2)	6.1
<i>Hydrangea hortensis</i>	2.46(5)	4.5(7)	...
Tomato (<i>Solanum esculentum</i>)	3.68(1)	5.3(3)	...
<i>Solanum pseudocapsicum</i>	3.70(1)	5.4(2)	...
<i>Manihot</i> sp.	4.02(1)
<i>Psidium</i> sp.	5.30(1)	7.8(2)	10.3
<i>Quillaya braziliensis</i>	6.94(2)	7.4(4)	19.4
<i>Gomphocarpus</i> sp.	6.96(1)	8.0(1)	17.6
<i>Acacia juncifolia</i>	11.43(2)	14.2(2)	...
<i>Hakea saligna</i>	12.47(1)	12.0(1)	16.8
<i>Erica</i> President Felix Faure.....	13.12(1)	12.0(1)	...

by some measurements of dye penetration made on plants growing outdoors. As the observations were made on material which was very variable as regards age and size and under varying conditions of exposure all the data will not be presented. Measurements made in the afternoon of August 26th with the temperature 18°C ., relative humidity 75 per cent and the soil very wet showed considerable dye penetration in shoots of Gooseberry, White Currant, *Hydrangea hortensis*, *Cytisus scoparius*, *Rhododendron ponticum*, *Berberis aquifolium*, *Taxus baccata*, and *Sambucus nigrus*. Measurements made on the same species during the morning of August 28th following about fourteen hours of continuous heavy rain, with the wet and dry bulb thermometers both reading 11.7°C ., showed a dye penetration of 4 cm. in *Cytisus scoparius*. In all the other species it was less than 1 cm. and was probably nil; but, owing to the fact that the stem is pierced below the surface of the dye solution in the plasticine cup, penetrations of less than 1 cm. cannot be determined readily because of contamination of the outer surface of the stem by the dye. This evidence of variation in the tension of the tracheal contents suggests that this may prove a useful method of studying the water relations of plants in the field (Warne, 1941), especially since we have here evidence that the tensions undergo marked changes and, with many species, disappear completely when water supply is good and transpiration nil for long periods, the rate of disappearance varying from species to species and the tension persisting longer in some species (e.g. *Cytisus scoparius*) than in others.

The data for the osmotic pressure of the cell sap of the leaves shown in the table vary from plant to plant. In general the tendency to develop a considerable tension in the tracheae accompanies a high osmotic pressure of the sap. In every case the estimated value for the tension is less than the osmotic pressure of the sap expressed from the leaves.

Discussion.—It is now possible to discuss these results in so far as they affect the general water relations of the plant. The generally accepted view of the mechanism of transpiration (Stiles, 1936) is that evaporation of water is either through the cuticle or into the intercellular spaces of the mesophyll, and that water vapor diffuses out through the stomatal pore and a gradient of suction force is set up between the evaporating surface and the tracheae, with the result that there is a flow of water from the tracheae to the evaporating surface. There is no *a priori* reason why water should pass from the tracheae to the evaporating surface. It will do so only as long as a suction force gradient is maintained. The greater the suction force exerted by the tracheal contents the less readily will water flow to the evaporating surface and the more will the water at the evaporating surface be withdrawn into the intercellular spaces of the cell wall, thus greatly increasing the resistance to evaporation. According to this view we can look upon the whole of the living cells as constituting a semi-permeable membrane sepa-

rating the evaporating surface from the tracheae. We cannot, however, neglect the fate of the living cells. If the latter are to remain turgid, they must be kept supplied with water. They can only remain turgid as long as the maximum suction force exerted by the non-flaccid cells is not exceeded by the suction force of both evaporating surface and tracheal contents. If the suction force of the evaporating surface is greater than the suction force of the tracheal contents and both are greater than the suction force of the living cells, water may pass to the evaporating surface through the living cells without maintaining the latter in a condition of full turgidity. Since even in a wilted leaf water continues to be transpired from the evaporating surface, we can assume that the maximum suction force of the evaporating surface is greater than the suction force of the tracheal contents, and the suction force of the latter is considered to be due mainly to the tension under which the liquid in the tracheae exists. So to maintain the living cells of the leaf in a condition of turgidity, their suction force must be greater than the suction force of the liquid in the xylem vessels and tracheids. It is to be expected that the maximum suction force of the leaf will be closely related to the osmotic pressure of their cell sap. On drying, shrinkage of the cell may occur before a fully flaccid condition is attained, so that the maximum suction force attainable may be considerably in excess of the osmotic pressure; there is also the possibility of rapid changes in composition of the sap, resulting in an increased osmotic pressure. Nevertheless, it is probably safe to assume that the osmotic pressure of the cell sap is a fair measure of the relative maximum suction forces which the cells of different plants are able to exert. Reference to table 4 shows that in all cases examined the osmotic pressure of the cell sap of the leaves exceeded the estimated tension of the tracheal contents. This accords with the observation that during these experiments no wilting of the leaves was observed. The data give a clue to the significance of the high osmotic pressures often found for sap of xerophytic plants. It was formerly considered (Fitting, 1911) that a high osmotic pressure of the leaf cell sap in itself depressed transpiration, but both Renner (1915) and Wisser (1914) consider the depressing effect of high osmotic pressures to be negligible. According to the views given above it is the suction force of the tracheal contents, due mainly to their tension, which will exert a depressing effect on transpiration rate, the significance of the high osmotic pressure of the leaf cells being that it allows the development of a considerable degree of tension in the tracheal contents without causing a flow of water from the living leaf cells to the tracheae. At the same time the tension facilitates passage of water from the living cells of the root to the tracheae. As long as the suction force of the tracheal content exceeds the suction force of the soil water, absorption is facilitated. The significance of the tension is at least twofold. It overcomes root resistance and facilitates water absorption and at the same time

reduces transpiration, and, as long as the tension does not exceed the maximum suction force of the non-flaccid leaf cells, it can do this without causing wilting of the leaf. The tensions disappear during periods of good water supply and low transpiration, and so probably the existence of the tension can be taken to indicate the existence of some slight internal water deficit, which, however, is prevented from becoming serious by the action of the tension itself on absorption and transpiration. We have here a self-regulating mechanism for preventing the development of a serious internal water deficit, but the efficiency of the mechanism is limited by the level of the osmotic pressure of the leaf cells. It is precisely in those plants whose cell sap has a high osmotic pressure that there seems to be the greatest tendency for a considerable tension to develop during periods when conditions favor high transpiration, and this, in itself, is likely to postpone the time when a serious deficit of water in the leaves occurs.

SUMMARY

The water conductivities of the stems of a variety of plants have been determined, and it has been shown that the conductivity per unit area of foliage supplied varies according to the species, the lowest value being given by *Ruscus aculeatus*.

An estimate of the pressure needed to raise water to the cladodes of *Ruscus* at a rate sufficient to cover the transpiration losses gave a value so low as to indicate that, even in this extreme case, stem resistance is unlikely to offer a serious obstacle to the upward movement of water to the transpiring cladodes.

Liquid exuded from a cut stump is derived from the xylem, but the rate of exudation from the stump rarely equals the rate of transpiration from the whole plant. It is concluded, therefore, that, in the intact plant, water is transmitted through the root system at a rate in excess of the rate at which a cut stump exudes liquid. It is necessary, therefore, to look upon the root system as constituting a resistance to the flow of water from the soil to the transpiring shoot.

Measurements have been made of the rate at which the stump of a well watered potted plant exudes liquid when subjected to a negative pressure. This rate of exudation has been expressed as a per-

centage of the rate of transpiration from the same plant, and the exudation rate expressed in this way varies considerably from species to species, the highest values being obtained for succulents and mesophytes and the lowest for xerophytes. The exudation rates obtained in this way give a measure of root resistance. A high exudation rate indicates a low root resistance and *vice versa*.

It is concluded that, in the intact plant, root resistance is overcome by the suction force exerted by the solution in the xylem and that this suction force is contributed to largely by the tension of the tracheal contents.

Estimates of the tension of the tracheal contents of intact potted plants are given. These estimates are based on a knowledge of the rate of exudation of a decapitated stump, when subjected to a known negative pressure, and of the transpiration rates of intact plants.

Data relating to direct observation of the relative tensions of the tracheal contents of plants are presented and show satisfactory agreement with the estimates mentioned in the above paragraph.

Values for the osmotic pressure of the sap expressed from the leaves of some of the plants used are given.

The estimates of the tension in the tracheae vary from 1.21 atmospheres for *Pelargonium sanicifolium* to 13.12 atmospheres for *Erica*, President Felix Faure. In general, under conditions favoring rapid transpiration, high values for the tension are found for xerophytes and low values for mesophytes and succulents. The tensions are reduced or disappear when plants are exposed to conditions in which transpiration is nil and the water supply good.

The tendency to develop a high tension in the tracheae may be advantageous in that it facilitates water absorption and tends to restrict transpiration.

The significance of a high osmotic pressure of the leaf cell sap is that it allows the development of a high tension in the tracheae without the latter causing a flow of water from the living leaf cells to the veins. A high tension in the tracheal contents will assist in the maintenance of a favorable water balance as long as it is accompanied by a high osmotic pressure in the leaf cells.

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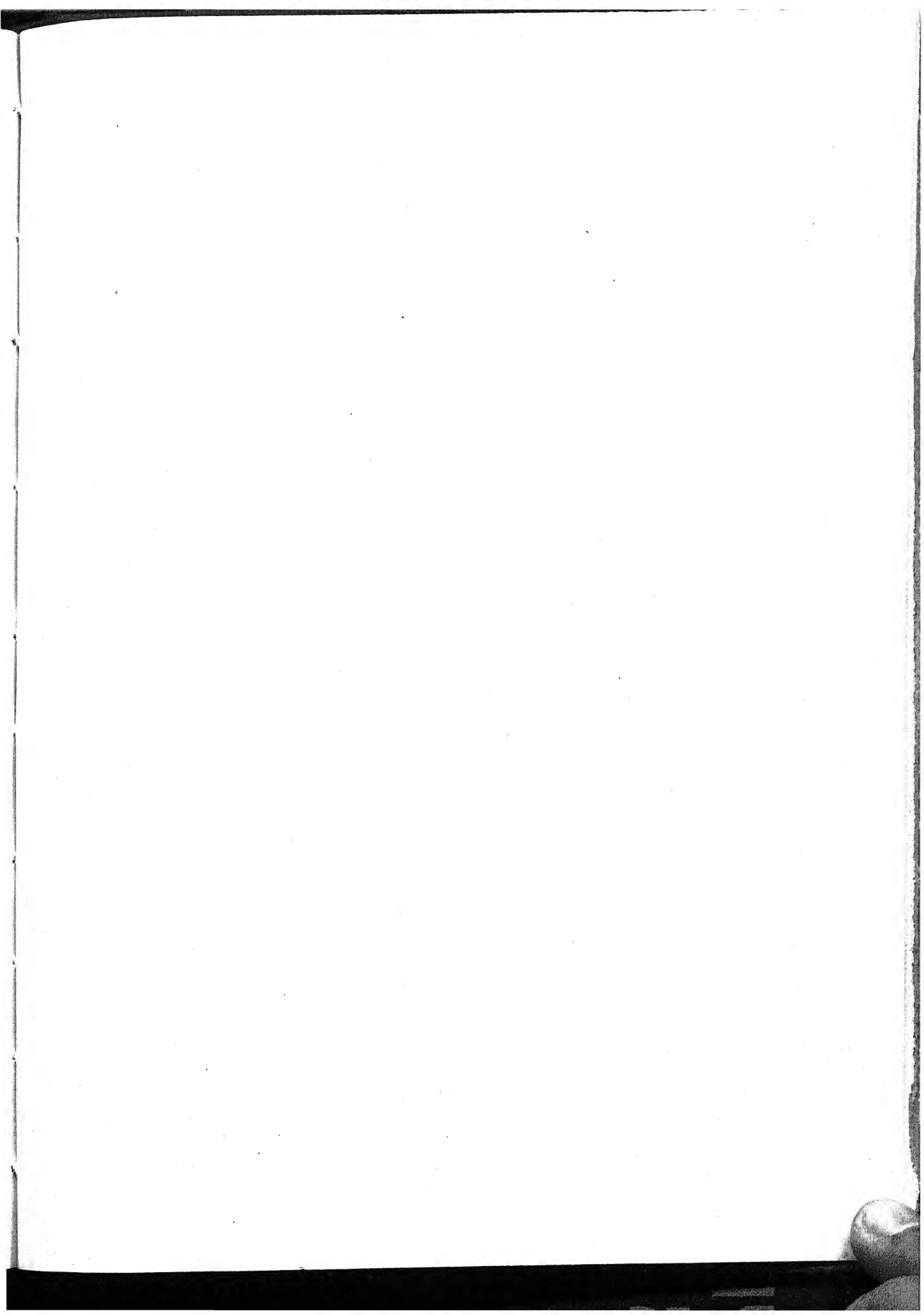
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ANDERSON, LEWIS E., and HENRY I. KOHN, Duke University, Durham, N. C. *The effect of certain sulfa-drugs on root elongation.*—Young seedlings of peas, corn, and radish were grown by immersing primary roots in various concentrations of sulfanilamide, sulfathiazole, sulfapyridine, and sulfadiazene. Root elongation was measured after one to seven days. Both roots and solutions were analyzed for drug content and some roots were examined cytologically. Sulfanilamide and sulfathiazole inhibit root elongation to a degree somewhat proportionate to the drug concentration of the culture solution. Sulfanilamide is a relatively more potent inhibitor of root growth than sulfathiazole. Inhibition by both drugs can be abolished by the addition of *p*-amino benzoic acid to solutions containing the drug. The amount of *p*-amino benzoic acid required to abolish a fixed amount of inhibition depends upon the ratio between added inhibitor and added *p*-amino benzoic acid. Inhibition is brought about by a decrease in the rate of cell division. Low concentrations of both sulfanilamide and sulfathiazole stimulate root growth as do low concentrations of *p*-amino benzoic acid alone as well as when mixed with low concentrations of either drug. High concentrations of *p*-amino benzoic acid itself likewise inhibit root growth. Roots grown in saturated solutions of sulfapyridine and sulfadiazene are stimulated or not affected apparently because of the relative insolubilities of these drugs as compared with sulfanilamide and sulfathiazole.

AVERY, GEORGE S., JR., JULIUS BERGER, and BARBARA SHALUCHA, Connecticut College, New London, Conn. *Accumulation of auxin in maize kernels during ontogeny.*—Corn kernels were assayed for their auxin content at successive stages in their maturity. The extraction method used was that developed in 1940-41 for the rapid and total extraction of auxin and auxin precursor from corn endosperm. The precursor is an, as yet, unidentified compound which upon alkaline hydrolysis yields indoleacetic acid. The precursor always constitutes the major portion of the total auxin obtainable at any time. There is little or no auxin present at time of pollination, but accumulation begins immediately afterward, reaching its peak in about 3 weeks. At their peak auxin

content, kernels contain 30 to 50 million TDC per gram dry weight (equivalent to 0.3 to 0.5 mg. indoleacetic acid). There is a marked drop in auxin content as the kernels approach dormancy, characteristic of all varieties studied. No relationship is apparent between auxin which accumulates in kernels, and the vegetative vigor of the plants producing them (inbreds vs. hybrids); nor is there any evidence of a relationship between polyploidy and auxin content. Kernels with sugary endosperm are consistently higher in total auxin than those with starchy endosperm.

BAILEY, I. W., Biological Laboratories, Harvard University, Cambridge, Mass. *Morphology and relationships of the Winteraceae.*—The Winteraceae is a remarkably homogeneous family as regards the morphology of its flowers, pollen, nodal anatomy, and wood structure. It exhibits a combination of morphological characters that is indicative of relatively remote relationship to such ranalian families as the Magnoliaceae, Schizandraceae, and Trochodendraceae.

BALL, ERNEST, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. *Growth in sterile culture of the shoot apices of Tropaeolum majus and of Lupinus albus.*—The shoot apices and subadjacent tissues of these plants have been grown in sterile culture for the purpose of gaining an understanding of the developmental potentialities of the embryonic and differentiating tissues. The shoot apex of *Tropaeolum* may grow in culture and produce a plantlet several centimeters long that consists of stem, leaves and adventitious roots. The shoot apex of *Lupinus* may produce a short shoot when grown in culture, but adventitious roots do not form under the conditions of the experiments. The young, differentiating tissues immediately behind the shoot apices of both *Tropaeolum* and *Lupinus* undergo a generalized growth in culture and produce a mass of tissue. The manner of growth of these tissues in culture indicates that the center of embryonic development in these plants is restricted to the shoot apex and does not extend to the subadjacent tissues. This is evidence of the physiological differences between the shoot apex and the tissues below it. Reorientations of these shoot apices with respect to the force of gravity did not change their polarity. In inverted or horizontal orientations, these shoot apices grew normally and produced short

¹ Because of a request from the Office of Defense Transportation, dated November 20, 1942, the meetings were not held on these dates but were postponed indefinitely.

shoots, the oldest ends of which then curved in a manner that tended to return the buds to the upright position.

BALL, ERNEST, and EDGAR J. BOELL, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and Yale University, New Haven, Conn. *Respiration of the shoot apices of Lupinus albus and Tropaeolum majus*.—The rates of oxygen consumption of the shoot apices and of subjacent, differentiating tissues of *Lupinus* and *Tropaeolum* have been measured with the Cartesian diver ultramicrorespirometer in order to obtain information on the relationship between metabolic rate, dominance and embryonic development. In *Lupinus*, a plant having apical dominance, a gradient in respiratory rate exists along the apico-basal axis. The relatively high respiratory rate of the shoot apex is apparently due to fundamental physiological dissimilarities between it and the differentiating subjacent tissues where lower rates of respiration prevail. In *Tropaeolum*, which lacks apical dominance except during early seedling stages, the respiratory rate of the shoot apex is lower than that of the immediately subjacent region, and there are pronounced decreases in respiratory rate in tissues farther down the shoot. There appears to be a correlation between the respiratory rates of parts of the axis of *Lupinus* and its anatomical gradients, but in the axis of *Tropaeolum* no such correlation exists. The respiratory rate of the shoot apparently has no correlation with its function as a center of embryonic development. It is not possible to state whether the high rate of respiration found in a dominant shoot apex is the cause or the result of dominance.

BANFIELD, WILLIAM G., and IRENE H. STUCKEY, Rhode Island Experiment Station, Kingston, R. I. *Extent of intercrossing among Agrostis species*.—Preliminary study shows correlation of chromosome number with morphological characters in *Agrostis* species. There is as yet no definite evidence whether *A. alba* and *A. tenuis* are distinct species or variants of the same species.

BARGHOORN, ELSON S., JR., Amherst College, Amherst, Mass. *The occurrence of marine cellulose-destroying fungi*.—A new series of marine fungi has been isolated during the course of investigations on the structural and chemical changes in the decomposition of submerged wood. The fungi are of very common and wide-spread occurrence along the North Atlantic coast. Pure cultures of 12 species have been obtained from wooden test blocks which had been continuously submerged for six to ten months in the sea. Several additional forms have been isolated from specimens of decaying piling and cordage collected in Massachusetts harbors. The organisms thus far determined belong to the *Pyrenomyces* and to several groups of the *Fungi Imperfecti*. In many cases the occurrence of abundant perithecia has facilitated the solution of taxonomic and cultural problems. Histological and microchemical examination of infected wood and cordage shows that the fungi bring about the enzymatic

hydrolysis of cellulose in the thick secondary walls of the fibers. Plant materials attacked by these organisms exhibit a marked deterioration involving loss of cellulose and concomitant reduction in tensile strength. The physiological activity of these fungi on a wide variety of substrata is now being determined.

BERGER, JULIUS, and GEORGE S. AVERY, JR., Connecticut College, New London, Conn. *Auxins and certain dehydrogenases of the Avena coleoptile*.—The ability of auxins to control certain phases of plant growth is well known, but their mechanism of action is still unknown. The object of the present study was to isolate dehydrogenases in cell-free extracts of the *Avena* coleoptile, auxin test object, and to investigate *in vitro* the effect of synthetic auxins on these enzymes. Considerable amounts of malic, ethyl alcohol, isocitric and *l*-glutamic acid dehydrogenases were found in the extracts. Fumarase and aconitases were also present. Succinic dehydrogenase was not found in the extracts, which suggests, with other findings, that the usual four-carbon dicarboxylic acid cycle is not operative in the *Avena* coleoptile. Naphthaleneacetamide and indoleacetic, naphthaleneacetic and indolebutyric acids had no accelerating effects on the activity of the malic acid dehydrogenase. The fumarase and alcohol dehydrogenase activities were likewise not increased by indoleacetic or naphthaleneacetic acids.

BERRY, WILLARD, Duke University, Durham, N. C. *Pleistocene plants from South Carolina*.—From Wilson's Landing, Santee River, South Carolina, a Pleistocene flora of some 55 species has been found. The specimens consist of extremely well preserved leaves and seeds. These remains accumulated in cool water swamps but indicate a climate about like that of today in the area. They are all species which grow in the region today.

BLOCH, ROBERT, Yale University, New Haven, Conn. *Cellular competence, differentiation and pattern in meristems of Monstera deliciosa*.—Leaves, petioles and air roots were used in studying differentiation and pattern in surface tissues during primary development and in formation of cork, absciss and wound tissues. Natural and induced meristems from which surface structures are regenerated are of the storied type; the character of the elements differentiated is dependent on the stage of development of the organ and to some extent on external conditions. In very young organs primary cell types may be completely redifferentiated (epidermal cells in leaves), while in old and less reactive parts cells may only divide and suberize; mostly, however, derivatives of the secondary ground parenchyma meristems differentiated in a uniform manner, resulting in a surface structure with a simple pattern, which consisted of thin-walled suberized cells toward the surface, thick-walled lignified sclerotic cells underneath, and thin-walled parenchyma cells in the innermost region. Evocation and character of this pattern appear associated with conditions near the outer surface, but could

also be induced toward necrotic surfaces and centers in inner regions of petiole and air root. The pattern is very common in plant tissues adjacent to external and internal surfaces. As part of the general pattern it is often differentiated in primary development, for example, as hypodermal pattern in air roots of *Philodendrons*, under certain conditions also in those of *Monstera deliciosa*. If regions thus differentiated were experimentally removed the pattern was reconstituted by secondary meristems from the ground parenchyma and structural regeneration ensued.

BOKE, NORMAN, Johns Hopkins University, Baltimore, Md. *Development of the leaf and areole in Opuntia cylindrica*.—The leaf in *O. cylindrica* is terete, pointed, and about 3 mm. long. In its initiation and early development it resembles a typical leaf. However, since it forms no marginal meristem, it produces no lamina and is thus equivalent to the axis of a laminate leaf. A constriction developed at the base produces early abscission. An axillary meristem appears early on the enlarged and persistent leaf base. This begins to produce spines, glochids, and trichomes while the leaf is still immature. These structures are formed asymmetrically about the meristem and with it constitute the areole, which may be regarded as a modified axillary bud. The differences between spines and glochids seem to be quantitative. Glochids, however, are produced on the adaxial side of the areole apex whereas the larger spines are not. Both structures arise from several cell layers, and both grow from a basal intercalary meristem; however, neither contains any vascular tissue, although vascular tissue differentiates to their bases. Every epidermal cell within the limits of the areole, excepting those on spines, on glochids, and on the areole apex, produces a uniseriate trichome. These trichomes therefore appear in dense masses completely surrounding all other structures. Only basal trichome cells undergo divisions. Although spines and glochids appear to be the much reduced equivalent of leaves, further comparative studies are needed to solve the problem of homologies.

BONNER, JAMES, California Institute of Technology, Pasadena, Calif. *Observations on riboflavin and pantothenic acid in tomato plants*.—Concentrations of riboflavin and pantothenic acid in tomato plants growing in sand culture were determined under various circumstances. For assay of the two vitamins use was made of the micro-biological methods using *Lactobacillus casei*. With both substances a concentration gradient from top to bottom of the plant appeared to obtain, and this gradient was similar to that earlier reported for thiamin. When stems or petioles of the tomato plant were girdled by steaming, pantothenic acid appeared to accumulate above a basal girdle whereas riboflavin showed no such accumulation. The rate of accumulation of pantothenic acid under these conditions was greater than that of riboflavin but smaller than that of thiamin.

BROWN, ALLAN H., University of Rochester, Rochester, N. Y. *The unique respiratory system of Micrococcus candidus cohn*.—The respiratory system of *Micrococcus* was investigated by the use of the inhibitors, CO, KCN, NaN₃, NH₂OH, and NaF. Young broth cultures were washed free of nutrient and suspended in phosphate buffer containing glucose. The effects of the poisons were determined by manometric measurements of oxygen consumption. 3.3×10^{-2} M. NaF had no inhibitory effect. Apparently the course of glucose desmolysis does not follow the conventional Meyerhof pathway. CO-O₂ mixture in the ratio 19/1 produced no effect either in the light or in the dark and 1×10^{-2} M. KCN inhibited only 21 per cent of the respiration. This is strong evidence that cytochrome oxidase is not functioning. Oxygen consumption was inhibited 70 per cent by 3.3×10^{-3} M. NH₂OH and over 95 per cent by all concentrations of NaN₃ down to and including 1×10^{-4} M. With many tissues cyanide and azide exert much the same effects; this is the only biological material which has been shown to have a respiratory mechanism which is especially azide sensitive yet essentially cyanide resistant. As such, the respiration of *M. candidus* must be considered unique.

BRUMFIELD, ROBERT T., Harvard University, Cambridge, Mass. *The number and arrangement of initial cells in root meristems of Crepis capillaris and Vicia faba*.—Certain types of chromosome rearrangements induced by X-rays are passed on through subsequent cell divisions to all the descendants of the affected cell. Since the same chromosome change is not likely to occur in two adjacent cells, such rearrangements offer a means of "tagging" a single cell and determining the kind and extent of tissues that it produces. Primary root meristems in germinating seed of *Crepis capillaris* and *Vicia faba* were subjected to X-radiation and then allowed to grow for three weeks. During this period the meristem was replaced by descendants of initial cells present at the time of raying. After the growth period the primary tips were studied cytologically. Study of chromosome morphology showed many of the roots to be chromosomal chimeras. Most of the chimeras were sectorial ones, the same aberrant karyotype being found in a wedge-shaped sector including root cap, epidermis, cortex, and central cylinder. In most of the chimeras the sectors having the aberrant karyotype amounted to about one third of the root, and in one case three sectors of about equal extent were present. These results suggest that the whole root develops from but few cells, probably three, at the extreme tip of the root. These are arranged in such a way that sectorial chimeras are frequently formed while periclinal ones occur rarely, if ever. Sectorial chimeras would not be expected if the root meristem consisted of "histogens" as described by Hanstein.

BURKHOLDER, PAUL R., Yale University, New Haven, Conn. *Vitamin deficiencies in yeasts*.—Growth-responses were determined for thirty-eight

kinds of yeast cultured in a chemically defined medium with varied supplements of vitamins and liver extract. Under the conditions of the experiments, fifteen kinds showed marked requirements for thiamine, none for riboflavin, twelve for pantothenic acid, six for nicotinic acid, thirty-six for biotin, five for inositol, and six for pyridoxine. Growth of all yeasts was augmented by addition of liver extract to the medium containing the seven vitamins. Some of these organisms may be useful in microbiological assays for vitamins. Growth of *Saccharomyces oviformis* in an enriched medium is proportional to the dosage of pyridoxine up to approximately 0.001 gamma per ml. of culture fluid. The requirement of this yeast for pyridoxine appears to be rather specific inasmuch as a number of pyridine compounds related to vitamin B₆ were found inactive.

BURR, H. S., Yale University, New Haven, Conn. (Introduced by E. W. Sinnott). *Potential differences and fruit form in cucurbits*.—Electrometric methods have been so successful in unraveling fundamental biological problems that it has seemed worthwhile to investigate the relationship between the relatively steady state standing potential and pattern of organization in living systems. Such a relationship has been described in the amphibian egg and has now been extended to the ovary of races of *Cucurbita Pepo*. Pot-grown plants of three of these were studied: (1) a race with elongate fruits, about three times as long as wide; (2) a race with essentially isodiametric fruits, and (3) a race with disk-shaped fruits, about twice as wide as long. Potential differences have been determined by continuous recording over periods of time varying from 15 minutes to many hours. Fruits varying from 10 mm. overall length to 70 or 80 mm. were included. In all three fruits, over-all lengthwise potential differences were determined and transverse potential differences through the greatest diameters. It was found that in the long type, the lengthwise potential difference was seven times the transverse. In the round type, it was twice the transverse, and in the flat type from one to one-half times. These ratios of potential difference are independent of size. Radial asymmetries were also reflected in the potential pattern. In all dimensions the potential gradient in millivolts per millimeter was, in round numbers, one millivolt per millimeter. This preliminary study suggests a significant relationship between the field forces in the fruit and the form which it ultimately assumes.

CARABIA, J. P., New York Botanical Garden, New York, N. Y. *Endemism in the flora of Cuba*.—Cuba is rich in endemic species, but this is true only for two regions, the Pinar del Rio in the west and Oriente in the east. The rest of the island has the common vegetation of the West Indies. The chief families of endemics are Rubiaceae, Euphorbiaceae, Bignoniaceae, and Compositae. In Pinar del Rio they grow on a very old calcareous soil; in Oriente the endemics are on the Sierra Madre and represented on two types of soil: (1) on igneous vegetable

mold, the vegetation being close to that of Hispaniola; (2) on limonite, where the vegetation is most peculiar and has no close affinities.

CHANEY, RALPH W., University of California, Berkeley, Cal. *The record of local diversity in the Bridge Creek flora*.—The Bridge Creek flora from the John Day Basin of Oregon represents a middle Tertiary forest in which the redwood was a dominant tree. Collections and specimen counts from several localities have yielded a total of more than thirty-two thousand leaves and fruits, of which *Sequoia langsdorfi* and other fossil species with living equivalents in coastal California make up nearly eighty per cent. The remainder have equivalents now occupying regions with summer rainfall, in eastern North America and eastern Asia. There is a marked variation in representation between localities, and at one locality the percentage of certain species may vary greatly within short distances horizontally and vertically. *Cercidiphyllum crenatum*, a characteristic member of the East Asian Element, is well represented only at one locality (Dugout Gulch), and even here it may be rare or absent at certain pits. Such irregular occurrence is consistent with the observed occurrence of its living equivalent, *C. japonicum*, in Asia. *Fagus pacifica*, *Castanea orientalis* and *Tilia oregona* of the East American Element also vary greatly in abundance, as is the case with their living equivalents in the mixed deciduous forests of Kentucky. Suggestive of topographic control is the predominance of *Sequoia langsdorfi* at the Twickenham locality, where it makes up seventy-eight per cent of the total, and appears to indicate an essentially pure stand on a valley flat. The abundance of the small-leaved, evergreen oak, *Quercus clarnensis*, at Crooked River, where redwood makes up only three per cent, suggests greater slope exposure, possibly on the leeward side of a climatic barrier. Such quantitative data provide a basis for a better understanding of the composition and environment of Tertiary forests.

CHEADLE, VERNON I., Rhode Island State College, Kingston, R. I. *The origin and certain trends of specialization of the vessel in the Monocotyledoneae*.—The metaxylem in all available parts of 320 species in 35 families were investigated. Characteristic tracheids have great length, are angular in outline, have inconstant width of walls in transverse section when the wall is thick, and have no or very slight development of an end wall. Vessels have originated from such tracheids. Those vessel members nearest to the tracheids in these characters have scalariform perforation plates with over fifty bars on very oblique end walls. Those vessel members which are least like tracheids have simple perforation plates on transversely placed end walls. Tracheids are the most primitive upright conducting cells in the xylem and vessel members with short length, simple plates on transverse end walls, oval to circular outlines in cross section, and constant width of cell walls at all degrees of thickness are the most specialized. Vessel members with scala-

riform plates are intermediate in specialization. Those with the largest number of bars are the most primitive, while those with the smallest number are the most highly specialized among such vessel members. In essential features, both the (1) origin and the (2) lines of specialization of the vessel in the primary xylem of the Monocotyledoneae parallel those of the vessel in the secondary xylem of the Dicotyledoneae. It seems clear that vessels have originated independently in these two groups of the Angiosperms.

CHENEY, RALPH HOLT, Long Island University and the Brooklyn Botanic Garden, Brooklyn, New York. *China tea substitutes in the New York area.*—More than twenty-five species which are hardy in the New York area may be utilized for the preparation of beverage tea. Taxonomically, they represent a wide diversity within the Angiospermae. Many of them are palatable and harmless in terms of human physiology. Others are stimulatory in effect. Some are definitely undesirable both in taste and in action. A few are palatably pleasant but surprisingly deleterious otherwise. War transportation has reduced drastically the United States importation of approximately 100,000,000 pounds of China tea annually. Collection and private growing of tea substitutes has increased rapidly during the current year. The availability of acceptable species in eastern nurseries and in their native habitats has been investigated. Products of a single species and taxonomically mixed tea substitutes can be purchased on the New York Market.

CHRYSLER, M. A., and W. G. MCINTYRE, Rutgers University, New Brunswick, N. J. *The morphological nature of the photosynthetic organs of certain bladderworts.*—*Orchyllium Endresii*, a terrestrial bladderwort from Costa Rica, is particularly favorable for study because it has fairly well developed vascular tissues. The underground stem and the inflorescence axis have essentially the same stelar structure, characterized by many scattered phloem groups. The leaf-like organ has a stele almost exactly like that of the inflorescence axis. The vascular elements of the "leaf" arise from the stele of the stem as a concentric structure, i.e., a stele. In the bract subtending each pedicel, however, are found 3–5 collateral bundles arising separately from the stele of the axis. It is inferred that the so-called leaf in *Orchyllium* is a cladode. It is probable that the same is true of the small blade-shaped organs of species of *Utricularia*.

CHURCH, GEORGE L., Brown University, Providence, R. I. *A cytological and morphological approach to the species problem in Glyceria.*—In an examination of the somatic chromosomes of the North American species of *Glyceria*, it is found that *G. pallida*, *G. neogaea*, *G. pauciflora*, and *G. erecta* are distinct in having a diploid complement of fourteen large chromosomes. Correlated features include acuminate lodicules, lanceolate, erose-tipped paleas and rippled walls of the epidermal cells. The above species stand apart from the rest of the genus

and have some floret details more in common with other genera with a basic number of seven, such as *Poa*, *Festuca*, *Puccinellia* and *Fluminea*. The other members of the section *Hydropoa* have small chromosomes in multiples of ten; truncate or spatulate lodicules; lanceolate or ovate, notched paleas; smooth walls in the epidermis and two rather than three stamens. The somatic complements of these species are: *G. striata* 20, *G. elata* 20, *G. canadensis* 60, *G. obtusa* 40, and *G. melicaria* 40. In an intermediate position in the section is *G. grandis* with 20 medium size chromosomes; lanceolate, erose-tipped paleas; truncate lodicules and only slightly rippled epidermal walls. The section *Euglyceria* has small chromosomes in multiples of ten, lodicules shortened to a somewhat thickened and more or less continuous ridge, and obovate, winged, bidentate paleas. Diploid species ($2n = 20$) include *G. borealis* and *G. Cookei*. Tetraploid species ($2n = 40$) include *G. septentrionalis*, *G. acutiflora*, *G. occidentalis* and *G. leptostachya*. A tetraploid form of *G. septentrionalis* is reported from Nova Scotia, thus extending the range of this species. *G. fluitans* is represented by a diploid form ($2n = 20$) in the upper Sacramento Valley, whereas the Pacific and Atlantic coastal forms are tetraploid.

CLAUSEN, ROBERT T., and CHARLES H. UHL, Cornell University, Ithaca, N. Y. *The taxonomy of the subgenus Gormanina of Sedum.*—The species of *Sedum* grouped in the subgenus *Gormanina* seem to represent a natural evolutionary series divisible into two sections, both restricted to the Pacific Mountain System of western North America. The section *Rosulata*, comprising two species, appears to be the primitive section. The section *Eugormanina*, comprising four species, exhibits various advanced characteristics. The chromosome number, with one exception, is $2n = 30$. The exception is *Sedum oregonense*, of which the only plants so far studied are hexaploid. *Sedum obtusatum*, *S. laxum*, and *S. spathulifolium* are each divisible into subspecies which exhibit geographical or ecological distinctness as well as the morphological differences which are the basis for their recognition. Although we recognize only six species, previous workers have described sixteen species for the same plants which we assign to this subgenus. Further, these species have been placed in three different relationship groups, even with one subspecies in one genus and another subspecies of the same species in another genus. Finally, Section *Oreganica*, recently described and included in subgenus *Gormanina*, should be removed from *Gormanina* on a basis of cytological evidence and gross morphology.

COMMONER, BARRY, and DANIEL MAZIA, Queens College, Flushing, N. Y., and University of Missouri, Columbia, Mo. *The mechanism of auxin action.*—The effect of indole-3-acetic acid and the four-carbon dicarboxylic acids on the rate of salt accumulation and water absorption by aerated potato tuber slices and *Avena* coleoptile sections was studied. Optimal concentrations of auxin produced a marked stimu-

lation of salt and water absorption. Both processes are a function of the auxin concentration, following the usual optimum curve closely parallel to each other. The optimum auxin concentration for both salt and water absorption by potato slices is 10–20 mgm. per liter; for *Avena*, 1 mgm. per liter. In hypertonic sucrose solutions auxin prevents the normal loss of water by potato slices, and in the presence of KCl causes water to be absorbed and the tissues to become turgid. This fact contradicts the wall extensibility theory of auxin action (proposed by Heyn *et al.*) since such slices do not have the initial turgor required by this theory. The presence of the four-carbon acids appears to be necessary for the auxin effect, thus confirming similar observations on the growth effects of auxin. It is concluded that the active driving force which causes cell enlargement is the accumulation of salt in the cell sap, since this process induces the absorption of water and increase in cell volume. Auxin influences cell enlargement through its effect on salt accumulation. The energy required by salt accumulation is probably derived from the metabolic activity of the four-carbon acid hydrogen transport system. Auxin appears to play an important part in this process.

COUCH, GLENN C., and ELROY L. RICE, University of Oklahoma, Norman, Okla. *Conjugation in a species of Desmidium*.—A collection of conjugating *Desmidium* was made in Southeastern Oklahoma (McCurtain Co.) in the fall of 1941. Although there is little doubt but that the species is *D. Grevillii* (Kutz) Debarry the type of conjugation exhibited by these specimens is quite different from that previously described. The conjugating cells in the filament do not dissociate, and the smooth walled spores develop in the receptive gametangia. Conjugating filaments over two hundred cells long were observed.

CROSS, GEORGE L., University of Oklahoma, Norman, Okla. *Further studies of the shoots of the Taxodiaceae—Athrotaxis and Taiwania*.—The cellular patterns in the shoot apices of *Athrotaxis* and *Taiwania* are similar to those of other genera of the Taxodiaceae. At the summit of each apex there is a single tier of apical initials which divide periclinally as well as anticlinally; lateral derivatives of the apical initials differentiate into a well defined protoderm; basal derivatives of the subapical initials become subapical mother cells; and derivatives of the subapical mother cells differentiate into a ring or cylinder of peripheral meristem and a core of pith mother cells. The apices of *Athrotaxis* are comparatively small (65–120 microns in diameter, measured in a plane equidistant from the shoot tip and the axil of the youngest leaf) and are hemispherical as seen in longisection. On the other hand, the apices of *Taiwania* are more massive (90–160 microns in diameter) and appear parabolic in longisection.

CROSS, GEORGE L., University of Oklahoma, Norman, Okla. *The shoot apices of the sequoias*.—The shoot apices of *Sequoia* and *Sequoiadendron* are similar to those of other genera of the Taxodia-

ceae. The apical meristem in each genus consists of a tier of apical initials, a flanking protoderm, a group of subapical initials, a peripheral meristem and a core of pith mother cells. This description is not in accord with those of Strasburger (1872) and Douliot (1870) for *Sequoia sempervirens*. The apical meristem of *Sequoia* is greater in diameter than that of *Sequoiadendron*. This difference in size is a result partially of differences in cellular number; however, statistical analyses show that the nuclear diameters, minor dimensions and major dimensions of the subapical mother cells of *Sequoia* are from twenty to thirty per cent greater than those of *Sequoiadendron*, and it is clear from this that differences in cellular size are involved also. The nuclei and cells of *Sequoia* exhibit greater variations in size than do those of *Sequoiadendron*. It is assumed that the differences in the shoot apices of the two genera are a result of polyploidy in *Sequoia*, and it is suggested that the determination of nuclear size, from "smears" or other preparations, may prove to be useful as a preliminary method of separating diploid and polyploid individuals.

CURTIS, OTIS F., and PALMER J. WASLIEN, Cornell University, Ithaca, N. Y. *Diurnal changes in food content of hay and other crops*.—Cutting alfalfa late in the afternoon has given a product containing 10 to 24 per cent more actual dry matter per unit area of field and 78 to 140 per cent more total starch and sugar than is found in matched areas cut early in the morning. When expressed as percentage of fresh weight the gain during the day or loss during the night is likely to be still greater than when expressed on an absolute basis. The original content and the amount of gain is influenced by the weather conditions preceding as well as during the experimental period. The data clearly indicate that the time of day that forage crops are cut may have a significant effect on their food value.

DALE, E. E., Union College, Schenectady, N. Y. *Dominance effects on floral pattern in a multiple-allelic series in Salpiglossis*.—The five normal or primary corolla lobes in *Salpiglossis* flowers usually maintain their identity in extra-lobed flowers and thus the location of the extra lobes can be determined. By designating the major corolla lobe 1 and the rest clockwise 2, 3, 4 and 5 an inbred strain of "extra-lobed" showed that 66 per cent of the flowers bore an extra lobe both in region 1–2 and 5–1. In 20 per cent, extra-lobing occurred at only one point, 9.6 per cent in region 1–2 and 10.4 per cent in 5–1. The remainder were chiefly multiple extra-lobed flowers in which the location of some of the extra lobes was uncertain. Thus the chances were 86 per cent that an extra-lobed flower would have its extra lobe or lobes immediately adjacent to lobe 1. In crosses, "extra-lobed" x "normal" gave 1.7 per cent of "extra-lobed" to 98.3 per cent normal flowers in F_1 , "extra-lobed" x "notched" 2.9 per cent "extra-lobed" to 97.1 per cent "normal," and "extra-lobed" x "cut" 85.6 per cent "extra-lobed" to 14.4 per cent "normal." "Extra-lobed," therefore,

was dominant only to "cut." A different result was obtained in the cross "extra-lobed" x "appendaged." Here the F_1 progeny showed 1.3 per cent of extra-lobed flowers, 14 per cent "normal" and 84.7 per cent in which one or both halves of lobe 1 were "notched." As far as observed, the remaining lobes (2-5) of the notched-lobe 1 flowers were "normal." Thus "extra-lobed" tends to produce a specific modification of the corolla pattern and gives three distinct types of effect in crosses with other members of the multiple series.

DIMOND, ALBERT E., and JAMES G. HORSFALL, University of Nebraska, Lincoln, Nebr., and Connecticut Agricultural Experiment Station, New Haven, Conn. *Chemical synergism*.—When a new compound is formed in a chemical reaction, its toxicity will usually differ from that of the original components. This fact led to the hypothesis that synergism and antagonism often result from chemical reaction of the components with each other. The hypothesis was tested by allowing two compounds to react, and assaying them and the product, chemically and biologically. Synergism and antagonism were proportional to concentration of the new product. A fungicidal system displaying synergism is CS_2 and dimethyl amine, yielding dithiocarbamic acid; one displaying antagonisms is ZnO plus mercaptobenzothiazole, yielding the zinc salt. In general, chemical synergism may result from (1) direct reaction, (2) reaction catalyzed by the living organism, or (3) reaction of one component (a) with a component (x) to form a product (ax) that is reactive with the second component (b). In each case the reaction product is the agent which determines toxicity. Conclusions: (1) In synergism, new compound formation may occur. If so, efficiency of synthesis of the active material can probably be improved. (2) Chemical synergism involving three components will be rare as compared with synergism involving two components.

DODD, JOHN D., Columbia University, New York, N. Y. *Three dimensional cell shape in the carpel vesicles of Citrus grandis*.—Internal cells from the carpel vesicles (juice sacs) of grapefruit were examined in the living condition. Cell walls were stained lightly with neutral red. Records were kept by making a careful drawing of each cell. In order to insure completely impartial selection the data were not tabulated and summarized until 100 cells had been drawn. Results showed an average of 13.85 faces per cell. The range in number of faces was from 9 to 18. The largest number of any one type was 22 cells each with 14 faces. Of the rest 39 cells had more than 14 faces and 39 had less. The number of edges per face varied from 3 to 8; 0.8 per cent were triangular; 25.9 per cent were quadrilateral; 41.6 per cent were pentagonal; 23.6 per cent were hexagonal; 7.0 per cent were heptagonal; and 1.1 per cent were octagonal.

DORF, ERLING, Princeton University, Princeton, N. J. *Late cretaceous-early tertiary floral changes in the Rocky Mountain region*.—A study

of the fossil record indicates (1) that from early Late Cretaceous to the end of Late Cretaceous time floral changes in the extensive lowland plains of the present Rocky Mountain region took place at a slow, uniform rate; (2) that at the end of the Late Cretaceous relatively rapid and far-reaching changes occurred, eliminating many of the "ancient" subtropical Cretaceous species, genera, and perhaps even families; even more marked changes occurred in the animal kingdom at this same time, including the extinction of the dinosaurs and the ammonites; (3) that the more modern, temperate vegetation of the early Tertiary (Paleocene) was quickly established over wide areas. An explanation of this rapid floral change is sought in one or another, or combination, of the following theories: widespread unrecognizable hiatus between rocks of Late Cretaceous and early Tertiary age; insufficient collections from critical beds; the effects of diastrophism on the vegetation; drifting of continents; sudden invasion; geographic changes in lands and seas; and changes in solar radiation.

EIGST, O. J., University of Oklahoma, Norman, Okla. *A comparative study of mitosis in diploid and tetraploid species*.—A comparison of the rate of mitosis in cells of diploid and tetraploid species was made through a study of the division of the generative cell of *Polygonatum biflorum* (diploid $2n = 20$) and *P. canaliculatum* (tetraploid, $2n = 40$). Three replicates of pollen cultures were kept in a germinating chamber for four, five, and six hours, respectively, and then fixed and analyzed for mitotic activity. Diploid and tetraploid pollen samples were planted on the same slide in separate locations; hence the comparisons of mitotic rate were made upon the two cytological types developing under the same conditions, for any given replication. Percentages of pre-metaphases and post-metaphases of the generative cell were used to calculate rates of mitosis. At four hours, mitosis was already underway in the diploid cells; however, the tetraploid did not show indications of division. There were greater numbers of post-metaphasic stages in four, five, and six hour replications among diploid, than tetraploid. The accelerated rate of mitosis is correlated with earlier flowering and more rapid maturation in the diploid as compared with the tetraploid. This method for determining mitotic activity of diploid and tetraploid cells offers opportunity for further comparative work with diploid and tetraploid material.

ELIAS, MAXIM K., University of Nebraska, Lincoln, Nebr. *Structure of grass and its evolution*.—The fundamental feature in the organization of grass stems is the arrangement of their vascular skeleton into nodes and internodes in such a manner that at each node a radially symmetrical circle of vascular bundles enters the leaf sheath. In ontogenetically early types of stems there is a single ring of vascular bundles in the internode. The closed, radially symmetrical sheath is apparently primitive and the more common split type is prob-

ably derived from it. Although the leaf of grasses differs greatly from that of *Equisetum* their basal parts are essentially similar and may be considered compound vegetative organs which are made of webbed small single-veined elementary leaves. If the two plant types did not originate from a common ancestral stock this similarity presents a striking case of homoplasticity. In the flowering glume or lemma of the late Tertiary Stipeae all parts find their homologues in the corresponding parts of the typical vegetative leaf of living grasses. But some of these parts are reduced and even suppressed in various representatives of living Stipeae. Because both lemma and vegetative leaf in modern grasses are adapted correspondingly to functions of flower protection and seed-dispersal, and of assimilation, their differences apparently increased with increase of adaptation. The leaf of the ancestral grasses may be expected to be less specialized.

EYSTER, H. C., University of South Dakota, Vermillion, S. D. *Adsorption: the mechanism of enzymes, sulfanilamide, antagonism, vitamin c, and selenium poisoning*.—Just as the adsorptive capacity of finely ground activated charcoal for methylene blue is decreased by ethyl alcohol, ether, chloroform, sodium barbital, sulfanilamide, and saponin, so it appears that the activity of enzymes is reduced similarly by the same narcotics. The study involved the action of diastase on soluble starch at 25°C. It is concluded that enzymes do not act in a manner that can be explained by the law of mass action, since it was found that 1 M cane sugar reduced the action of diastase on soluble starch just as much as 1 M glucose did, and 1 M KNO₃ reduced the activity about twice as much. Sulfanilamide reduces the adsorptive capacity of charcoal markedly and only mildly affects enzyme action. Hence, it appears that sulfanilamide acts bacteriostatically by reducing cellular and colloidal adsorption, possibly for food, while at the same time only slightly influencing the metabolism of the host. *p*-amino benzoic acid, although toxic in that it reduces cellular and colloidal adsorption, nullifies the bacteriostatic effect of sulfanilamide by *antagonism*. Data are available to show that the Cu-Ca, Ca, Na, and *p*-amino benzoic acid-sulfanilamide-antagonisms are partial or complete cancellations of each other's toxicity in decreasing adsorption when acting alone or in an unbalanced mixture. Vitamin C (50 ppm) was found to increase by 23 per cent the adsorptive capacity of charcoal for methylene blue at 25°C. The reaction is not due to the reducing capacity of vitamin C. Selenium (100 ppm) as sodium selenite was found to reduce adsorption of charcoal 35 per cent, and to be successfully antagonized by arsenic as sodium arsenite.

FERNALD, M. L., Gray Herbarium, Cambridge, Mass. *An amateur detective in a grassfield*.—Study of type of *Erianthus brevibarbis* Michaux shows it to be not the coastal plain species usually so called, but a rare plant of the Mississippi Basin.

FERNALD, M. L., Gray Herbarium, Cambridge, Mass. *An amateur detective in the carrot patch*.—*Angelica triquinata* described by Michaux from "Canada" proves to be the Alleghenian *A. Curtisii* Buckley, the supposed region "Canada" being a misprint for "Carolina."

FLINT, LEWIS H., Louisiana State University, Baton Rouge, La. *The reappearance of a large oscillator in Louisiana*.—In 1938 a large *Oscillatoria* was found at several places in Louisiana, the material being the subject of a brief report to this Section at the Richmond, Va., meeting of that year. During subsequent years unsuccessful attempts were made to find the plant. During the past summer the *Oscillatoria* of the 1938 collections was found to be plentiful at one of the original sites. This reappearance suggested that the plant might be of the interesting type or group designated as ephemeral. Some of the distinctive characteristics are restated.

FLINT, LEWIS H., Louisiana State University, Baton Rouge, La. *Note on photosynthetic activity in seeds of the spider lily*.—Experiments conducted with seeds of the spider lily afforded evidence that the reserves supplied to the ovule following fertilization were utilized in the development of accessory structures, including chlorophyllous tissue. The mature seeds did not take up water upon immersion, nor germinate while immersed, yet when such conditions permitted photosynthesis there was an appreciable accumulation of starch in the embryo. It appeared that both the development of the embryo and the germination of the seed were made possible largely through the photosynthetic activity of the chlorophyllous tissue developed from the integuments.

FRENCH, C. S., ELOISE NEWCOMB, and M. L. ANSON, University of Minnesota, Minneapolis, Minn., University of Chicago, Chicago, Ill., and Rockefeller Institute, Princeton, N. J. *The evolution of oxygen from illuminated suspensions of chloroplasts*.—Further experiments on the O₂ evolution (the Hill reaction) by illuminated spinach chloroplasts suspended in sucrose solution containing ferric iron, oxalate iron and ferricyanide iron have shown that there is a pH change due to the binding of a basic constituent or to the production of an acid which is not carbon dioxide. The phosphate previously used as a buffer can be omitted thus allowing a greater range of iron and oxalate concentrations to be used without tying up the iron as a phosphate complex. In phosphate-free solution we have found that up to about 0.5 M the O₂ evolution increases with an increase in oxalate, but at higher concentrations the rate becomes independent of the oxalate concentration. The ferricyanide which is essential to prevent the accumulation of ferrous iron during the reaction is itself also an inhibitor. There is, therefore, an optimum concentration of this substance at about 0.015 M, above or below which the rate of the reaction decreases.

GAISER, L. O., McMaster University, Hamilton, Ontario, Canada. *Pollen mother cell division*

in *Martynia louisiana* Mill.—Following the first nuclear division of meiosis no cell plate is formed and the spindle fibers disappear. A dense perinuclear zone of lipoidal content that alternately surrounds the nucleus and achromatic figure (perispindle zone) becomes divided and surrounds each daughter nucleus. After second division no fibers persist and areas of lighter staining, presumably more fluid cytoplasm separate the four daughter nuclei. Centripetal furrows, which are believed to be invaginations of the plasma membrane, advance through these while callose material, such as secreted about the mother cell protoplast is also secreted in the furrows as cytokinesis progresses. Thus the mechanics of furrowing is accomplished independent of any fibers.

GALSTON, ARTHUR W., University of Illinois, Urbana, Ill. *The extraction and analysis of intact oat chloroplasts*.—Intact chloroplasts were isolated from oat leaves by a technique previously described by Granick. Microkjeldahl analyses of entire leaf and chloroplast fractions indicated that at all stages of growth, in both green and chlorotic leaves, the chloroplasts contain 30–40 per cent of the total leaf nitrogen. Since about 80 per cent of chloroplast nitrogen is protein nitrogen, the chloroplasts must synthesize large amounts of the protein of the leaf. Chloroplasts transferred to hypotonic solutions gradually swelled and burst, indicating the presence of a semipermeable membrane around each plastid. Complete precipitation of plastids from an aqueous suspension was obtained by the use of a heat-labile agglutinin extracted from castor bean seeds. The apparent isoelectric point of the agglutinin was 4.6, indicating that it might be the albumin ricin, which also possesses erythrocyte-agglutinating properties.

GIER, L. J., William Jewell College, Liberty, Mo. *Root systems of Burley tobacco*.—A preliminary study of the roots of the Burley tobacco in loess soils shows fewer but more widely distributed roots than reported for Bright Belt tobacco.

GILLY, CHARLES L., New York Botanical Garden, New York, N. Y. *The phylogenetic development of the inflorescence in Carex and related genera*.—Three sorts of developmental trends have been operative in the production of the diverse inflorescence types of the tribe Cariceae. On the basis of a combination of inflorescence morphology with geographical distribution, a phylogenetic series is postulated. The significance of the floral structure in the group is considered, and the relationship of *Carex* and associated genera to the remainder of the family Cyperaceae is discussed.

GILLY, CHARLES L., New York Botanical Garden, New York, N. Y. *Floral structure and classification in the subfamily Mimusoideae (Sapotaceae)*.—Study of western hemisphere Sapotaceae has revealed that terminology of floral parts in the subfamily Mimusoideae (Hartog) Lam. seems to need reinterpretation. Correlative studies of eastern hemisphere members of the subfamily have been

necessary to further indicate the apparent evolutionary trends in the group. A partial result of this study has been the amplification of the subfamily to include certain genera previously placed in subfamilies Sideroxyloideae (Dubard) Lam. and Madhucoideae Lam. The relationship of the subfamily Mimusoideae, as herein constituted, to the remainder of the family is briefly discussed.

GLEASON, H. A., New York Botanical Garden, New York, N. Y. *Plant life of the Pacaraima range*.—The Pacaraima Mountains have long been noted for their numerous endemic species. The relationship of these endemics indicates that the flora has been derived partly from Andean and Amazonian sources, probably in comparatively recent time, but also contains plants showing distinct affinities with species of southern Brazil and West Africa. These facts contribute to new ideas on the origin and migrations of the American flora in general.

GORDON, ROBERT B., State Teachers College, West Chester, Pa. *Part of Rafinesque's herbarium discovered at West Chester, Pa.*—Examination of the century-old Darlington Herbarium in the possession of the State Teachers College at West Chester has brought to light more than 50 authenticated specimens from Rafinesque's collection. Historical records show that they were part of a collection of more than 1000 plants received by Dr. William Darlington from Elias DuRand, of Paris, in 1842. More than 20 specimens bear the original labels of the AUTIKON BOTANIKON and are among the few remaining illustrations of one of the rarest botanical works.

GRAHAM, E. R., and WM. A. ALBRECHT, University of Missouri, Columbia, Mo. *Nitrate adsorption by plants as an anion exchange phenomenon*.—Because of the importance given to the cation exchange from the clay of the soil to the root of the plant in what has been known as contact exchange and what has been demonstrated by means of calcium and hydrogen ion systems, the question naturally arises whether there cannot be anion change from the colloid to the plant in a similar manner. Experiments were carried out to determine the exchange capacity of a commercial product known as Amberlite. This colloid adsorbed the anion nitrates very readily. The exchange capacity of Amberlite used amounted to 160 M E of nitrate per 100 gms. of Amberlite in its moist condition, and to 319 M E when the Amberlite was dried at 100°C. This Amberlite was used as a carrier of the adsorbed nitrate. It was mixed into a subsoil that is very low in nitrogen and was used to grow corn plants. Potassium phosphate, 10 M E per three gallon jar of substrate, was used as a basic soil treatment in all trials. Three trials were completed. In the first were included the modified substrate, the substrate plus 15 gms. of Amberlite carrying adsorbed carbonate anion, and another treatment in which the Amberlite carried adsorbed nitrate ion. A second trial included two different amounts of calcium nitrate in solution and corre-

sponding amounts of the nitrate ion in the adsorbed condition. In the third trial nitrate was employed in solution and in the adsorbed form; both were subjected to a leaching treatment after the corn had grown for three weeks. The final crop was harvested at the end of six weeks. In the first case the Amberlite carrying the carbonate ion was without effect while the Amberlite carrying the adsorbed nitrate gave an effect almost exactly twenty times as large as that obtained on the Amberlite substrates without adsorbed nitrogen. When the crop growing on the Amberlite with nitrate was compared with a crop growing on the nitrate in solution, the results show that the adsorbed nitrate was as effective as the nitrate in solution. When the soils were leached, the leaching performance reduced the growth because of nitrate removal where it was applied in solution, but leaching was without effect as a reducer of crop growth where the nitrate was in the adsorbed condition. These results show that the nitrate ions in the adsorbed form are available for plant nutrition, and may be as effective as the water soluble nitrogen. Such results suggest that attention for purposes of plant nutrition may well go to the adsorbed anion as well as to the adsorbed cation.

GRAHAM, E. R., and WM. A. ALBRECHT, University of Missouri, Columbia, Mo. *Soil development and plant nutrition. II. Mineralogical and chemical composition of sand and silt separates in relation to the growth and chemical composition in soybeans.*—The recent demonstration that sands and silts, isolated from soils representing different climatic conditions, when treated with hydrogen clay, would effect the growth of soybean plants suggested that it would be worth while to characterize the chemical and mineralogical nature of these sands and silts as well as to determine the chemical composition of the plants grown. A rather complete analysis has been made. Methods for carrying out this type of analysis have been studied and developed. As a result of the various determinations it can be demonstrated that silts and sand containing the heavy feldspar fraction (Ca bearing feldspars) and a large suite of different minerals, will furnish nutrient cations for soybean plant growth, which result in soybeans of a high calcium and magnesium content. Also, the soybeans grown on the mineral-rich substrate fixed large amounts of nitrogen. Sand and silts containing quartz and potassium feldspars with a limited variety of minerals furnish few, if any, cations for soybean growth. The soybeans grown on the substrates poor in minerals fixed no nitrogen and contained very limited amounts of calcium and magnesium.

GREGORY, MARGARET P., The Blandy Experimental Farm, University of Virginia, Charlottesville, Va. (Introduced by O. E. White). *Chromosome investigations in the Aristolochiaceae.*—Approximately 15 species of *Asarum* native to North America and 30 species of *Aristolochia* from Europe and North, South, and Central America have been studied cytologically. In the South and Central

American group only diploid species ($n = 7$) of *Aristolochia* have been found while in the North American species only tetraploids ($n = 14$) have been observed. Both diploid and tetraploid chromosome counts have been made in European species. *Asarum* differs from *Aristolochia* not only in chromosome number ($n = 13$) but also in chromosome morphology. Correlation between chromosome complement and systematic grouping is noted. Possible association of polyploidy with other features is considered.

GUNCKEL, JAMES E., Harvard University, Cambridge, Mass. *Certain dynamic aspects of shoot growth in Ginkgo.*—Ginkgo affords an excellent illustration of short shoot—long shoot relationships, in which either shoot expression is capable of change to the other. Such changes may occur normally, or may be induced artificially. Experimental evidence, considered in the light of available information, shows: (1) that decapitation of the apical portion of any terminal or lateral branch results in the change to long shoots of a limited number of adjacent short shoots; (2) that application of auxin in place of the decapitated apical portion will effectively inhibit the short shoots below. It seems, therefore, that variations in short shoot growth are directly referable to variations in the rate of auxin supply. The growth control of apical buds, whether as long or short shoots, is very complex. Several possible explanations are suggested: (1) Apical bud expression may be dependent upon secondary inhibition from adjacent lateral branches. (2) Short shoots may have an increased rate of destruction of the auxin formed. (3) A deficiency, e.g., of nitrogen, associated with reduced auxin and also reduced vegetative growth, may be limiting in short shoot growth. (4) Prolonged cold treatment, possibly with excessive dryness, releases short shoots from apical inhibition.

GUNDERSEN, ALFRED, Brooklyn Botanic Garden, Brooklyn, N. Y. *The relationships of the Hamamelidaceae.*—The structure of flowers and of flower buds of *Liquidambar* and other genera of Hamamelidaceae is compared with similar structures of Hydrangeaceae, Platanaceae, Betulaceae and Cornaceae. The resemblances are of interest in connection with early fossil representation in these groups. A diagram presents carpel and stamen structure of various families of dicotyledons.

HITCHCOCK, A. E., and P. W. ZIMMERMAN, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y. *Modified organs and absence of correlative inhibition induced by p-chlorophenoxyacetic acid in Kalanchoe daigremontiana.*—In plants treated around the stem with lanolin preparations of p-chlorophenoxyacetic acid (3.2 — 10 mg./g.) correlative inhibition was lacking with respect to axillary shoot growth and to the growth of plantlets. Leaves were greatly modified as to size, shape, marginal serrations, and character of both surfaces. At the fourth or fifth node above the treated region most stems terminated in a funnel-

shaped organ 10 to 30 cm. long, variously cleft, with part or all of the margin smooth, and lacking a midrib although the tissue resembled that of leaves. Similar organs also developed in the axils of leaves or as modified leaves, sometimes resembling Jack-in-the-pulpit flowers in shape. From or near the margin of a terminal funnel-shaped organ 13 plantlets continued to grow, and now at a length of 6 to 9 cm. and with leaves 6 to 8 cm. long (with full complement of secondary plantlets), the shoots appear normal except for size. On control plants the plantlets seldom reached a length of 1 cm. In addition to the modified organs, four to six axillary shoots continued an indeterminate growth typical of normal stems. Of the several phenoxy and benzoic compounds tested, only *p*-chlorophenoxyacetic acid caused consistently and to the same degree all of the responses described.

HITCHCOCK, A. E., and P. W. ZIMMERMAN, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y. *Structure of phenoxy compounds in relation to root-inducing activity*.—The root-inducing activity of phenoxy compounds varied with the kind, number, and positions of substituents in the ring and with the relative length of the side chain. Compared with β -indolebutyric and α -naphthaleneacetic acids the root-inducing activity on cuttings of horticultural varieties was as follows for the substituted phenoxy compounds: the monohalogen substituted phenoxy acids were less, 2,4-dichloro- and dibromo-phenoxyacetic acids equal or greater, and the corresponding 2,4-propionic and butyric homologs and 2,4,5-trichlorophenoxyacetic acid 10 to 100 times greater. The activity for monosubstituted phenoxyacetic acids was: (*Euonymus*) para > ortho > meta; Cl > I at the ortho position; Cl > NH₂ > NO₂ at the meta position; and Cl > Br > NH₂ at the para position; (*Ligustrum ovalifolium*) m-NO₂ > p-NH₂ > p-Br, p-Cl, m-NH₂, m-Cl, o-Cl (latter five inactive up to 32 mg./l.). For other phenoxy acids (*Ligustrum*) 2,4-dichlorophenoxypropionic > 2,4-dichlorophenoxybutyric > 2,4-dichlorophenoxyacetic; phenoxybutyric acid > phenoxypropionic acid > phenoxyacetic acid; and for chlorophenoxyacetic acids (or ethyl esters) 2,4,5-trichloro- > 2,3,4,6-tetrachloro- > 2,3,4,5,6-pentachloro- > 2,4-dichloro- > monochloro- (o, m, or p). Mixtures of three dichlorophenoxy homologs or of one dichlorophenoxy homolog and either or both β -indolebutyric and α -naphthaleneacetic acids were more effective than the individual compounds. Phenoxy compounds induced marked fasciation of roots at concentrations near and above optimum. Phenoxypropionic and phenoxybutyric acids stimulated basal callus growth on *Ligustrum* cuttings, but induced relatively few roots. The alpha forms of propionic and butyric phenoxy acids were used in all cases.

HODGE, W. H., Massachusetts State College, Amherst, Mass. *Some aspects of the vegetation of Dominica, B.W.I.*—Because of its rugged terrain, which has restricted agriculture, the natural flora

of Dominica has been well preserved. This is not true of the majority of the Lesser Antilles which are heavily cultivated. A study of the vegetation of Dominica can thus serve as an example of the type of vegetation which formerly occupied most of the volcanic Caribbees. Four principal types of vegetation are recognized: (1) the pantropical vegetation of the strand; (2) the xerophytic scrub forest of the leeward coasts—typical of elevations up to 1000 feet; (3) the broad-leaved, evergreen, montane rain forest occurring between the 1500 and 2500 foot elevations; and (4) the mossy elfin forest of the highest summits—above 3000 feet.

HOPKINS, MILTON, University of Oklahoma, Norman, Okla. *A taxonomic study of Polygonatum in eastern and central North America*.—A recent paper by O. J. Eigsti involving cytological studies on *Polygonatum*, made by the colchicine pollen-tube technique, indicates that speciation within the genus is obviously due, at least in considerable part, to polyploidy. Continued work this year has further confirmed this conclusion. The present systematic treatment shows that the glabrous *P. biflorum* (n = 10) is restricted to the old Appalachian area of North America and that *P. canaliculatum* (n = 20), also a glabrous plant, doubtless is a polyploid, closely related to *P. biflorum*, and which occurs to the north and west in great abundance. It also occurs frequently within the range of *P. biflorum*. The specific differences between these two entities are indicated chiefly by *gigas* characters which are quantitative rather than qualitative. Even in herbarium specimens, whose chromosomal counts cannot be made, these features are obvious. Therefore, a key on which to differentiate the two must be a highly artificial one. *P. pubescens* (n = 10) is clearly distinct from the other two species because of its pubescent leaves. The appearance of diploid clones within the range of *P. canaliculatum* is also noted, and this observation brings up the fact that polyploidy may not be the complete answer to the question of speciation. Breeding studies will be made in the future in the hope that they may cast additional light on the question.

HOSKINS, J. H., and A. T. CROSS, University of Cincinnati, Cincinnati, Ohio. *A consideration of several species of the fructification of Bothrodendron*.—Investigations on a large number of Iowa coal balls have disclosed some excellent, preserved, fragments of *Bothrodendron* fructifications. A review of the principal characteristics is undertaken in order to justify proper designation of species.

HOSKINS, J. H., and A. T. CROSS, University of Cincinnati, Cincinnati, Ohio. *The structure and affinities of a new species of Sphenophyllotachys*.—A portion of a petrified *Sphenophyllum* fructification found in an Iowa coal ball has been studied and is described as a new species. The specific distinction is based on the nature of the arrangement of the sporangia. Three unbranched sporangiophores of unequal length arise at slightly

different points near the base of the bract bears a single sporangium placed linearly on the surface of the bract. Thus in longitudinal section through the strobilus, three sporangia may be seen upon the adaxial surface of each bract. Affinities with *S. Dawsoni* are clear, but since we question the homogeneity and validity of that species, we cannot justify the inclusion of our specimen within it.

HULBARY, ROBERT L., Columbia University, New York, N. Y. *The three dimensional shape of cortical cells in the stem of Elodea*.—In the cortex of *Elodea densa* (*Anacharis densa* Victorin), the shape of cells in the internodes is influenced by the presence of large air canals. These cells are all elongated along the longitudinal axis of the stem and nearly every one abuts upon either 2 or 3 of the air canals that run the full length of each internode. The cells are regularly arranged in rows surrounding the air spaces. The average number of sides per cell for 3200 cells taken from 27 consecutive internodes, starting 6 mm. back of the stem apex, was 8.81. Of 500 cells studied more intensively, which averaged 8.85 faces, 195 were 8-sided with the others ranging from six sides to fourteen. There was a total of 1425 facets on the 500 cells and of these 64 per cent were quadrilaterals, 19 per cent were hexagons, and 13 per cent were pentagons. The average length of the cells studied varied from 45 microns in an internode 6 mm. back of the apex to 331 microns in the 27th internode or 17 cm. back of the apex, where the cortex is mature. The number of sides per cell remains fairly constant for each internode throughout the 17 cm. of *Elodea* stem studied. The average number of sides per cell for 100 cells in the first internode back of the stem apex is 8.85 and for 100 cells in the 27th internode 8.82. Apparently the shape of cortical cells of the internodes of *Elodea* is determined near the very apex of the stem—within the first 6 mm.—at the same time that the elongate air spaces are formed. Increase in cell size from one internode to the next does not result in an increase in the number of contacts per cell.

HUNT, KENNETH W., College of Charleston, Charleston, S. C. *Floating mats on a southeastern coastal plain reservoir*.—The plant succession in floating mats on a reservoir at Charleston, S. C., is described. It differs greatly from the familiar northern bog development, having *Jussiaea grandiflora* and *Achyranthes philoxeroides* forming the pioneer zone, and a variety of southern mesophytes occupying the interior. It is believed that this succession is unusual for the southeastern coastal plain, where due to the fluctuating water-table xeric shrub bogs normally develop. This may suggest what should be expected when reservoirs with stabilized water levels are constructed in the southeast. Data on the history and composition of the reservoir are supplied.

JOHNSON, MARION A., Rutgers University, New Brunswick, N. J. *Zonal structure of the shoot apex of Encephalartos*.—Six species of *Encephalartos*

have been examined. The shoot apex in all six species is broadly cone-shaped or mound-like and may terminate in a plateau about twenty cells in diameter. The summit is occupied by a zone of initiation which contributes to surface growth and to an underlying zone of central mother cells. The central mother cells are characterized by their large size, conspicuous vacuoles and complex cell pattern. Radiating files of active cells arise from this zone. The lateral files, together with the superficial layer and its periclinal derivatives, constitute a peripheral meristem. A rib-meristem, which produces the massive pith, is derived from the base of the central mother cell zone.

KARLING, JOHN S., Columbia University, New York, N. Y. *Two new genera of parasites and their bearing on the phylogeny of the lower fungi*.

—These genera parasitize the marine algae *Pyraliella* and *Ectocarpus* and occur in abundance in the vicinity of Beaufort, North Carolina. One of the parasites has an animal- or *Proteomyxa*-like type of nutrition (engulfing solid food particles and digesting them in vacuoles) and a life cycle identical to that of the *Plasmodiophorales* with anteriorly biflagellate heterocont zoospores. It combines thus the characteristics of the *Proteomyxa* and *Plasmodiophorales* and suggests that these two groups of lower organisms may be closely related. The other parasite is similar in structure and development to the olpidoid chytrids but has anteriorly uniflagellate zoospores which excludes it from the *Chytridiales* as this order is now generally recognized.

LANGDON, LADAMA M., Goucher College, Baltimore, Md. *On the structure and development of the cupule in the Fagaceae*.—The indehiscent 1-seeded fruit, tricarpetate flowers, and the special involucre are distinctive of the *Fagaceae*, with considerable debate as to the nature of the involucre, whether it is composed of the fused bracteoles of the little dichasium, or represents sterile scales of a condensed cone-like inflorescence, or is a wholly new outgrowth of the subfloral axis. Developmental and anatomical studies of species of different genera of this family, including *Quercus*, *Fagus*, *Castanea*, *Castanopsis* and *Nothofagus* emphasize the essentially uniform nature of this structure, varied as its external features may be, and reveal it as a ring-like overgrowth of the subfloral axis which more or less completely incloses the 1-3 flowered female inflorescence, and bears numerous acropetally developed scales, variously modified in the different genera. A noteworthy resemblance to the gland-bearing husk enveloping seeds of the *Lagenostomales* is seen in the gland-adorned segments of involucre enclosing the female florets and nutlets of certain species of *Nothofagus*.

LEONIAN, LEON H., and VIRGIL GREENE LILLY, West Virginia University, Morgantown, W. Va. *The "unknown factor" in the growth of yeast*.—"Substances of unknown nature present in liver and yeast extracts play an important role in promoting rapid growth." This was the conclusion of Williams, Eakin

and Snell who were the first to study the relationship of five B vitamins to the growth of yeasts. But now we have found that insofar as some ten strains of yeasts are concerned, this unknown factor is not qualitative in nature but quantitative. The liver or yeast extracts do not furnish new growth factors but merely increase the amount of the well known vitamins and thus bring about much greater yields. Williams et al. used 5 micrograms of inositol per ml, 0.04 micrograms of thiamin, 0.0001 micrograms of biotin, 0.006 micrograms of pantothenic acid, and 0.04 micrograms of pyridoxin. We increased inositol to 10 micrograms, thiamin to 0.1 micrograms, biotin to 0.001 microgram, pantothenic acid to 0.1 microgram, and pyridoxin to 0.1 microgram. This brought about sharp increases in the yield of all ten yeasts; addition of yeast extract failed to bring about significant increases. Now it should be possible to use yeasts in microbiological assays without fear of complicating, unknown factors.

LEVINE, MICHAEL, Montefiore Hospital, New York City, N. Y. *The effects of x-rays on "colchicine tumors" on the root tips of the common onion.*—The root tips of onion bulbs (*Allium cepa* var. *yellow globe* and var. *Brigham Yellow Globe*) immersed in .01 per cent solution of colchicine in tap water develop after 48 hours characteristic swellings, "colchicine tumors." The return of these bulbs to tap water is followed in 48 to 96 hours by the resumption of growth of the tips of the "tumors." The exposure of these roots to x-rays (900r, 1500r, or 3000r) prior to their return to water inhibits their growth. Cytological studies of the roots exposed to .01 per cent colchicine for periods from 18 to 140 hours and to the larger doses of x-rays show that these combined treatments impair permanently the ability of the cells to divide. "Tumors," twenty-four hours in water after colchicine and x-ray treatments fail to reveal any evidence of cell division. Later stages show lethal effects characterized by undifferentiated nuclear masses and absence of cytoplasm. The treated bulbs were kept in water for twenty or more days after treatments. Control studies of the effects of water, colchicine, and x-rays were made concurrently. These results suggest that colchicine and x-rays not only injure cells in the "metaphase" stage but also the nuclei in the resting stage. These results suggest further, that some spontaneous tumors of animals and cancer in man may respond more advantageously to x-rays when preceded by suitable doses of colchicine.

MANNING, WAYNE E., Northampton, Mass. *A leaf character for shagbark hickory.*—The leaves of young and mature trees of *Carya ovata* can be distinguished from the leaves of all other northeastern hickories by one or two dense tufts of hairs on the upper part of each serration; these are usually accompanied by a ciliation of single or fascicled hairs. On seedlings and small saplings of this species the leaf margins are almost uniformly ciliate as in those

of the mockernut, the king nut hickory, and very young pignuts.

MARIE-VICTORIN, FR., University of Montreal, Montreal, Canada. *A new American Dracaena.*—Only one true *Dracaena* was known in the Western hemisphere. A second species, collected several times, but unrecognized on account of its being sterile, grows in the province of Oriente, Cuba. It is a member of the *Pinus cubensis* association and its range is restricted to the serpentine rocks and iron soils of the pinelands of Moa. The species, which seems the closest approach to *Dracaena Draco* of the Canary Islands, is here described as *Dracaena cubensis*.

MARVIN, JAMES W., University of Vermont, Burlington, Vt. *Cell shape and cell volume relations in the pith of Eupatorium perfoliatum.*—The number of faces on compressed spheres of uniform size when aggregated to form polyhedra approximates 14. Matzke has shown that when spheres of different sizes are compressed the smaller have less than 14 and the larger more than 14 faces. Fifty small and fifty large cells from a mass of *Eupatorium perfoliatum* pith were selected and their shape determined from three-dimensional models. The small cells averaged 10.8 faces and the large cells 16.2 faces.

MATZKE, EDWIN B., Columbia University, New York, N. Y. *The role of interfacial tensions in cell shape determination.*—Three dimensional shapes occurring in individual bubbles in a mass of foam are compared with three dimensional shapes occurring in cells in undifferentiated tissues. Similarities and differences are pointed out, and the relative importance of such tensions in cell shape determination is analyzed.

MATZKE, EDWIN B., and ROBERT L. HULBARY, Columbia University, New York, N. Y. *Wood characters and species characters in the white pines of commerce.*—A study has been made of the pits of the ray parenchyma cells in contact with the tracheids in the northern white pine, *Pinus strobus*, in the western or Idaho white pine, *P. monticola*, and in the sugar pine, *P. lambertiana*. Data have been assembled on the size, shape, and area of more than 200 such pits. They are more commonly large and oblong in *P. strobus*, smaller and lemon-shaped in *P. lambertiana*, and intermediate in *P. monticola*. The range of variation is pointed out, and its bearing on the subject of species relationships is suggested.

MERRILL, ELMER D., Arnold Arboretum, Jamaica Plain, Mass. *Some aspects of Rafinesque's botanical work.*—A brief summary of Rafinesque's publications and steps now being taken to elucidate his work more thoroughly. He actually proposed in the neighborhood of 3000 generic names as new, infinitely more than any other botanist, for even Linnaeus utilized less than 1600. Only a small proportion of Rafinesque's names were recognized by his contemporaries and successors. A critical examination of all known Rafinesque botanical papers, issued between 1804 and 1840, indicates that there are

apparently between 1200 and 1500 Rafinesque plant names in all categories—genera, subgenera, and species—that have never been listed in our standard indices. It is proposed eventually to prepare and issue a comprehensive "Index Rafinesquianus" which is planned to include all plant names proposed by him, with reductions where possible.

MERRILL, E. D., Arnold Arboretum, Jamaica Plain, Mass. *American weeds in the old world tropics*.—A consideration of the periods in which weeds, together with economic plants, were originally introduced into the Indo-Malaysian region, their American sources, and an attempt to explain the Napoleonic ambitions indicated by many of these alien plants when once established in their new homes. There is no evidence available that would indicate any pantropical distribution of these pest plants previous to Magellan's voyage in 1520. In most parts of the Indo-Malaysian region the dominant weeds today are those species of American origin, the great centers of origin apparently being Mexico and the eastern bulge of Brazil.

METZNER, JEROME, Columbia University, New York, N. Y. *Recent studies of Volvox carteri (Stein)*.—This relatively rare species of *Volvox* has been described under various names by workers in widely separated parts of the world. The present study involves careful investigation into the details of the life cycle. A comprehensive photomicrographic record of the living organism and of its morphology was made. The development of a daughter colony and the germination of the oospore into a juvenile colony are illustrated and discussed.

MEYER, JAMES R., The Blandy Experimental Farm, University of Virginia, Boyce, Va. *Cytogenetic studies of Phlox*.—Eighteen species of *Phlox* from Eastern North America, involving 276 plants, have been studied cytologically. A random examination of named horticultural varieties and plants from the wild has shown: 76.7 per cent diploid ($2n = 14$); 20.7 per cent with from 1 to 13 small, centric fragment chromosomes ($2n = 14 + 1-13$ ff.), 1.9 per cent tetraploid ($2n = 28$), 0.4 per cent (1 individual) triploid ($2n = 21$), and 0.4 per cent with 16 chromosomes ($2n = 16$). The centric fragments do not seem important, since they may vary in number from none to 13 per cell within one plant without noticeably affecting either cell viability or the morphology of the plant. *Phlox Buckleyi* and 4 of the 24 *P. subulata* plants are tetraploid. *P. suffruticosa* Miss Lingard is a triploid. It was found that, within *P. subulata*, tetraploids do not always have larger cells and organs than do the diploids, but when a pure strain of *P. Drummondii* was colchicine-treated, the resulting tetraploid had larger cells and organs than the diploid. Idiograms and meiotic studies have shown that intra-specific and inter-specific differences in chromosome morphology and structure are common.

MILLER, HELENA A., Harvard Biological Laboratories, Cambridge, Mass. *Development of tissues in embryos of Phlox*.—The development of em-

bryos of *Phlox Drummondii* were studied from the first divisions of the zygote to the embryo of the mature seed. Sections of early proembryos show an axial symmetry and a cellular homogeneity. Sections of later proembryos show regions of varying staining capacities: a dark-staining central core of elongate cells, a surrounding region of light-staining cells and an enclosing potential epidermal layer. The axially symmetrical proembryos become bilateral with the appearance of the cotyledons on or about the seventh day. As the cotyledons expand from then to about the thirtieth day the above mentioned core of elongate cells becomes extended uninterruptedly into them as characteristic procambial leaf strands suggesting the procambial nature of the original core. The ground meristem of the earlier proembryo is also extended as the cotyledons develop. During this same time the outer layer of potential epidermis divides periclinally in the basal region forming the root cap. As the cap becomes complete from the fifteenth to the twentieth day the suspensor disintegrates and disappears. Interior to the root cap in the basal region, a hypocotyl meristem appears which by its activity extends the procambium, ground meristem, and epidermis of the hypocotyl. By the activity of a rib meristem at this hypocotyl meristem, the earlier central core of procambium becomes a cylinder. In the embryo of the mature seed, adaxial cells in the procambial strand of the upper half of the cotyledons have become mature protoxylem; meanwhile certain abaxial procambial cells in the basal region of the cotyledons show the characteristics of immature sieve tubes.

MILLER, LAWRENCE P., Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y. *Sugar components of β -glycosides formed in plants through treatment with chemicals*.—Many of the less common sugars that have been shown to be present in plants occur as components of glycosides. Naturally occurring glycosides are found only in certain species and are often of complex structure and identification of the sugar component is frequently difficult. Experiments in which plants were treated with chemicals containing an hydroxyl or potential hydroxyl group (such as ethylene chlorohydrin, *o*-chlorophenol or chloral hydrate) have shown that many species including some not known to contain glycosides normally, will form β -glycosides if furnished with chemicals which can serve as aglucons. Such glycosides have been obtained in crystalline form and identified through the preparation of the corresponding synthetic compounds from 15 species of higher plants belonging to 9 families. Frequently the same aglucon will unite with more than one sugar in the same species and even in the same plant organ. As would be expected, the sugar most commonly utilized for glycoside formation was D-glucose; however, with all the solanaceous species tested some gentiobioside was also formed, although gentiobiose was not previously known to occur in this family. Some of the species studied were able to withstand the absorption of surprising-

ly large amounts of added chemical without serious injury. As a result the glycosides comprised a major constituent, quantitatively, of the plant tissues produced. Tobacco leaves were obtained which contained up to 12 per cent on a dry weight basis of a mixture of β 2-trichloroethyl-D-glucosides and β 2-trichloroethylgentiobioside.

MURPHY, JOHN B., De Paul University, Chicago, Ill. *The influence of magnetic fields on seed germination.*—The influence of magnetic fields was studied on several different species of seeds in the process of germination. In the majority of cases the seeds showed a definite acceleration in germination and in several instances produced a more hardy type of plant than the plants not influenced by magnetic forces. The initial vigor often seemed to subside after the plant reached a growth of several weeks. Some of the plants influenced by the magnets reached maturity before the control plants. The permanent magnets used were steel magnets of varying forces. Electromagnets were also employed and a third type of magnet was derived from the field about wires through which an electric current flowed. The type of magnet used did not seem to be very important. The electromagnet was the least desirable because it affected the temperature of the soil. Some possible effects of the magnetic influence on cell activity were studied. The experiments are not conclusive but are suggested with the hope of stimulating a more extensive and complete study of the subject.

NEAL, LOUISE A., University of Colorado, Boulder, Colo. *Germination and growth of certain plant species as influenced by seed treatment with growth substances.*—The germination and later growth of eleven plant species as influenced by the application of growth-promoting substances to their seeds is reported in this study. The percentage of germination was increased in Penstemon, Verbena, Vinca, Antirrhinum, Petunia, and the Scarlet O'Hara morning glory by the use of Hormodin Powder No. 1. Mixtures of indole-3-acetic and α -naphthaleneacetic acids in dust form stimulated germination in the first three species mentioned above, and also in Cleome and the moonflower; the use of indole-3-acetic alone was favorable not only for these five species but also for Coleus, Petunia, and the blue Japanese morning glory. When only α -naphthaleneacetic was used germination was increased in but two species, Verbena and Vinca. Vegetative growth was stimulated and dry weight increased in snapdragons treated with all the chemicals with the exception of the mixture of indole-3-acetic and α -naphthaleneacetic acids (100 mg./l.); in barley, by all substances with exception of indole-3-acetic acid (10 mg./l.). Blossoming was hastened in snapdragons by seed treatment with indole-3-acetic acid in either solution or in the dust form; in Cleome, by the use of Hormodin Powder No. 1. More striking results in increasing germination percentage and vegetative growth were obtained from the use of mixtures of indole-3-acetic and α -naphthaleneacetic

acids in the dust form than by the use of the substances alone.

NEWCOMB, ELDON H., University of Missouri, Columbia, Mo. *A study of the respiration of the large oak apple gall.*—The galls produced by *Amphibolips confluentis* Harris on the leaves of Red Oak (*Quercus borealis* var. *maxima*) contain 370 times fewer cells per unit volume of tissue than do normal leaves during the month of May. The oxygen uptake per cell is also more than 40 times greater in the gall tissue. The respiratory quotients in both cases were unity. The respiratory rate of both gall and normal leaf tissue falls as the organs develop and ultimately loses its sensitivity to cyanide and azide. An insensitive residual respiration remains in both cases but the change comes earlier and more abruptly in the gall tissues. Both gall and normal leaf tissues carry on fermentation in the absence of oxygen, but the rate of carbon dioxide evolution per cell is many times greater in the former. Galls differ from normal leaves in producing appreciable amounts of organic acids which tend to accumulate in the tissues.

PENNEL, F. W., Academy of Natural Sciences, Philadelphia, Pa. *Zones of plant life in the Colombian Andes.*

PIERCE, JOHN H., New York Botanical Garden, New York, N. Y. *The status of the genus Lophotocarpus.*—For many years the name *Lophotocarpus* has been applied to United States plants of the family *Alismaceae* which actually belong to the genus *Sagittaria*. In recent literature an incorrect type species has been cited for the genus. If *Lophotocarpus* is to be maintained as a genus, the correct type species limits the application of the name to plants not found in the United States.

PRICE, WINSTON, and PAUL J. ALLEN, University of Pennsylvania, Philadelphia, Pa. *Studies on respiration and flow in the myxomycete, Physarum polycephalum.*—The Q_{O_2} , based on the fresh weight and on O_2 consumption determined with a Fenn respirometer, has a value as high as 23.0 with an average of 15.0, this rate remaining constant for 21½ days. The Q_{O_2} varies with the amount of tissue used. Below 30 mg. there is injury and the rate declines sharply with smaller amounts of tissue. The R. Q. is about 0.8. Anaerobic CO_2 production is 1/3 the aerobic, indicating the absence of a Pasteur reaction. There is only a small reduction in respiration when O_2 tension is reduced to 5 per cent, but below 5 per cent it drops off rapidly to a value of less than 10 per cent of normal at 0.3 per cent O_2 without stopping the protoplasmic streaming. The streaming is therefore independent of a very large percentage of the energy released in aerobic respiration. Furthermore, 60 per cent of the O_2 consumption is inhibited by M/1000 KCN, which has no effect on flow. CO up to 95 per cent has no effect on respiration except that due to the reduction in O_2 tension, indicating the absence of cytochrome oxidase or polyphenol oxidase. NaN_3 inhibits respiration to about the same extent as KCN. Azide, how-

ever, reversibly inhibits streaming in concentrations of M/1000 to M/3000. M/20 malonate and M/500 pyrophosphate do not inhibit respiration, indicating the absence of succinic dehydrogenase. NaF and ethyl urethane inhibit respiration only slightly. None of these four poisons affect flow. Experiments with iodoacetate are in progress. The respiration is indifferent to the addition of M/20 Na succinate, fumarate, malate, lactate and pyruvate. The plasmodium disintegrates immediately when Na citrate is added. Vitamins B₁, B₂ and C do not affect respiration, but it is increased on addition of 2 gamma of nicotinic acid /ml.

REED, CLYDE F., Harvard University, Cambridge, Mass. *Comparative morphology of spores in ferns and its relationship to taxonomy*.—A survey of the spores of over 4500 species of ferns was made, and a correlation of this and other morphological data, such as cellular, stomatal and tracheary, has revealed many new phylogenetic relationships, heretofore unrealized. This necessitates a reclassification of the ferns into a more natural sequence, to be published as a series of books: History and Philosophy of Fern Taxonomy; Morphology of Ferns; a Synopsis Filicum, with descriptions of all known species, varieties and forms; Phytogeography, with maps of the genera and families; Ecology of the Ferns, including physiological data on growth; Phylogeny of the Ferns, including evidence from the fossils; a new Index Filicum, to include citations to genera, species, varieties and forms. In spores, the characters of the perispore and exospore, the size and shape, the color and the nuclear relationship afford family, generic and often species differences.

REID, MARY E., National Institute of Health, U. S. Public Health Service, Bethesda, Md. *Biological interrelations of ascorbic acid and calcium*.—Results of studies with plants and animals from many laboratories suggest that ascorbic acid and calcium are closely involved in functioning at cell surfaces and in the formation of special types of intercellular substances. The evidence in support of this concept is summarized.

RICKETT, H. W., New York Botanical Garden, New York, N. Y. *Cymose and racemose inflorescences*.—Some so-called racemes originate with a terminal flower in the axil of whose prophyll arises a shoot which simulates a continuation of the main axis. A repetition produces a sympodium whose lowest flowers open earliest. Similar statements may be made about various "umbels," "heads," and "spikes." The order of flowering therefore seems unsatisfactory as defining types of inflorescences; the terms monopodial and sympodial are suggested in place of racemose and cymose. In some racemes the terminal flower is the first to open. Order of flowering has therefore no necessary connection with morphology. A branch terminated by a flower, beneath which similar floriferous branches may appear, is held to be primitive. From such a panicle

cymes, corymbs, racemes, and spikes may have been derived.

ROBERTS, EDITH A., Vassar College, Poughkeepsie, N. Y. *The structure of chloroplasts, chromoplasts, leucoplasts and carotin "crystals" and their relationship to cellulose "particles" and colloidal carbon*.—The photomicrographs published in 1940 show that chloroplasts are composed of units 1 micron in size and these are termed plastid granules. Work upon the chromoplasts, leucoplasts and carotin "crystals" show that they too are composed of units approximately 1 micron in size. These units are held together by protoplasmic strands forming the respective plastid bodies and they, in turn, are held by strands in the protoplasmic matrix. The granule bodies are themselves composed of smaller units—about 0.5 microns in size—termed *primary* granules, and these, in turn, are composed of still smaller units—about 0.25 microns in size—termed *secondary* granules. The secondary granules are composed of units about 0.1 microns in diameter—called *tertiary* granules. These units are made up of *quaternary* granules—0.04 microns in diameter, and again these are formed of 0.02 micron units or *quintary* granules. In leucoplasts filled with starch, the quintary unit is subdivided into *senary* granules—0.01 microns in diameter. In "cellulose particles" and in colloidal carbon, there is a unit comparable to this senary granule. The "cellulose particle" is shown to be composed of the same primary, secondary, tertiary quaternary, quintary, and senary granules. This senary granule is also in the size realm of the viruses and suggests a possible relationship. During this investigation, the carotin "crystals" were easily separated into the 1 micron granule unit, as were the sheets of carotin embedded in the walls of the carrot root. If "all types of coal represent stages in the progressive disintegration or carbonization of plant tissue," then it is quite possible that in the building of plant tissue there is a basic design and that the larger units—"cellulose particles" or granules—are a repetitive pattern of the lowest units or the smallest colloidal carbon particle and the granules or "cellulose particles" make up the plastids and tissues of the plant.

SATINA, SOPHIA, and A. F. BLAKESLEE, Smith College, Northampton, Mass. *Initiation and development of carpels determined by periclinal chimeras in Datura stramonium*.—Periclinal chimeras were used in studying development and structure of carpels which form a syncarpous pistil with axile placentation. Initiation and early development of the two carpellary primordia depends primarily on activity of the innermost third germ layer. At early stages the first and second germ layers are each only one cell thick. Two opposed concave carpel walls develop from the primordia. A portion of the central axis remaining within the carpel primordia grows continuously to form an elongated column. The septum and false septum with placental tissue differentiates from portions of the axis. Their early

growth depends on activity of the third germ layer. The upper regions where the carpel walls coalesce and unite with the septum are formed predominantly by cells from the second layer. This occurs in later stages. The larger basal part of the ovary continues to grow from the activity of the cells of the third germ layer. Terminal growth of carpel walls forms the style and stigma. Cortex of the style is formed from cells of the second germ layer; vascular system from cells of the third germ layer; and transmitting tissue in the style and stigma from cells of the first germ layer. Early development of the carpel suggests that it is axial in origin.

SINNOTT, EDMUND W., and ROBERT BLOCH, Yale University, New Haven, Conn. *Fiber development in Luffa, the sponge gourd.*—In a considerable number of lines of *Luffa cylindrica* and *L. acutangula* a study was made of the development of the anastomosing network of fibrous strands which gives these plants their economic importance. The lines were found to differ markedly not only in the size and shape of the fruit but in the character of the fiber system. Its strands vary from 0.1 mm in diameter to 0.5 mm or more. The network may be relatively loose or much more compact. The diameter of the peripheral system of strands—the “wall” of the sponge—varies from 2 mm to 8 mm in different lines. The outer strands tend to run transversely to the fruit axis and the inner ones parallel to it. Differentiation of strands begins very nearly in ovary development and the entire framework is usually established by the time of anthesis. Each strand possesses a small core of phloem elements, which in the larger ones are accompanied by a few xylem cells. The bulk of the strand consists of much elongated heavily lignified sclerenchyma fibers which are very difficult to macerate. These range from 700 micra to well over 3000 micra in length and from 25 micra to 50 micra in diameter. Lignification of fibers begins first in strands near the axis of the fruit. Fruits developing parthenocarpically after treatment with various growth substances show essentially the same fiber development as do normal fruits.

SMITH, A. C., Arnold Arboretum, Jamaica Plain, Mass. *Geographic distribution and speciation in the Winteraceae.*—As now constituted, the Winteraceae is a family of 6 genera and about 88 species, occurring in the Australasian and Malayan regions and also in America south of Mexico. The criteria used for generic delimitation in the family are discussed. The genus *Drimys* is an interesting illustration of bicentric-palaeantarctic distribution; the probable method of differentiation in *Drimys* in America is outlined.

SMITH, A. C., Arnold Arboretum, Jamaica Plain, Mass. *Vegetational zones of northern South America.*—The region under consideration is the portion of South America from the Amazonian plain and Bolivia northward, with an area of about 3,500,000 square miles. This region presents a vast diversity of habitats, with elevations up to more than

6000 m., with a great range of temperature, and with rainfall varying from essentially none to more than 300 inches per year. Probably about 50,000 species of flowering plants and ferns occur in this area, parts of which are botanically unknown. Altitudinal floristic zonation is obvious in northern South America; the arbitrary elevations of about 1000 m., 2500 m., 3500 m., and 5000 m. may be taken to represent the upper limits of the tropical, subtropical, temperate, and alpine zones respectively. In each of these major zones we find a multitude of minor divisions, dependent upon such factors as local conditions pertaining to rainfall, humidity, topography, and soil. The alpine meadows of the northern Andes are of especial interest, because of the pronounced endemism of their vegetation. It seems probable that only three major elements have entered into the composition of the flora of northern South America. By far the largest of these is a pantropical element which probably indicates a common, but remote, ancestry for the flora of South America and Africa. A second element, represented by certain temperate groups of plants suggestive of the flora of North America, has presumably travelled southward from a boreal center of dispersal. A third element indicates a relationship between the floras of South America and Australia, suggesting an ancient Antarctic connection.

STADLER, L. J., U. S. Department of Agriculture and University of Missouri, Columbia, Mo. *Gene action in anthocyanin synthesis in maize.*—Excised roots of certain strains of maize, cultured on agar with added mineral nutrients and glucose, synthesize anthocyanin. The genes A_1 , A_2 , and r^+ , or suitable alleles, are essential to the synthesis. With genotypes $a_1 (A_2 r^+)$ and $a_2 (A_1 r^+)$, an anthoxanthin pigment is synthesized instead of anthocyanin. Certain alleles of r^+ result in the synthesis of different quantities of anthocyanin, and with $r^s (A_1 A_2)$ no anthocyanin is produced.—The same 3 genes are essential for anthocyanin production in the vegetative organs of normally grown plants, except that r^+ is not essential in the presence of B . Genotypes $B (r^s A_1 A_2)$ and $r^+ (b A_1 A_2)$ produce the same anthocyanin, and in those tissues which may be pigmented by the action of either gene, the quantity of anthocyanin produced is in general greater with B than with r^{ch} , the r^+ allele most effective in plant pigmentation.—Excised leaf sections of genotype $r^{ch} (b A_1 A_2)$, floated on glucose solution, produce anthocyanin abundantly in epidermal cells. The amount of anthocyanin produced varies with the glucose concentration, and under the standard conditions used the reaction is sensitive to concentrations of the order of $10^{-3}M$. Various r^+ alleles differ in quantity of anthocyanin produced at a given glucose concentration. In plants capable of anthocyanin synthesis through the action of B instead of r^+ (genotype $B r^s A_1 A_2$) the excised leaf reaction is wholly negative, regardless of the concentration of glucose.—The excised leaf reaction is inhibited by dinitrophenol. Certain other sugars (fructose, galactose,

xylose, lyxose) and certain sugar derivatives (sorbitol, glyceric acid, glyceraldehyde, hydroxypyruvic aldehyde) may be substituted for glucose with positive effects, though in some cases with lowered effectiveness. None of these results in anthocyanin production in genotypes a_1 , a_2 , or r^2 .

STEWART, RALPH R., New York Botanical Garden, New York, N. Y. *The flora of Kashmir and its relationships*.—Kashmir is the northern-most part of India and stretches roughly from 32' to 36' north and from 74' to 78' east. Except for the famous Vale of Kashmir it is all mountainous, containing some of the most rugged country in the world. There is a semi-tropical flora in the foothills and there are all the intermediate steps to an alpine flora of great variety. No flora has yet been published but about 2,200 flowering plants and 100 species of ferns have been collected. The flora contains a number of diverse elements. Representatives of the desert flora which stretches east from Arabia are found in the Jhelum and Indus Valleys. The subtropical plants come from the southeast and some have a distribution which reaches to the Philippines. The temperate and alpine floras, as might be expected, have both eastern and western elements. Behind the main Himalayan Range there is hardly any rain and the flora is Tibetan or Central Asian. In addition there are quite a few endemic species.

STRUCKMEYER, B. ESTHER, University of Wisconsin, Madison, Wis. *Further studies on the micromincineration of plant material*.—Previous observations have shown that a difference in mineral residue exists in stems of vegetative and flowering plants. From these observations several problems arose which were investigated. Considerable difficulty was encountered in obtaining incinerated sections in which the tissue was not displaced or curled. After trial of numerous adhesives, "Nevillite 123" was found to be satisfactory as it prevented the thick walled material from shivering and shrinking. By the use of this adhesive the mineral inclusions retained their position during incineration. The use of either absolute alcohol, absolute alcohol and formalin, cellosolve, or dioxan as fixing agents showed almost no difference in the amount of mineral residue. Dioxan appeared to have some shrinking effect on the tissue. The incineration of vegetative stems from several different plants of a species showed a similar mineral pattern. A difference in the mineral pattern became apparent soon after plants were placed in an environment conducive to flowering. Identification of the minerals in the stems is being made with histological reagents employing micropipettes and a micromanipulator.

SVENSON, HENRY K., Brooklyn Botanic Garden, Brooklyn, N. Y. *Relation of the Galapagos flora to that of the mainland*.—Climatic conditions on the Galapagos Islands are practically identical with those of the dry coast of Ecuador. A number of species supposedly endemic to the Galapagos are found on this coast, and some of the widespread species show a similar type of stunted growth in the Galapagos Islands and on

the desert mainland. This climatic area includes the Galapagos Islands, the southern coast of Ecuador, and the northwest coast of Peru. The flora of the Galapagos Islands is also connected with the dry areas of western Mexico, northern Colombia, and the West Indies.

TRYON, R. M., JR., Dartmouth College, Hanover, N. H. *Dynamic phytogeography of the fern genus Doryopteris*.—*Doryopteris* is primarily a New World genus. Of the 26 species only 5 occur in the Old World and these are not considered here. Species evolution in the genus probably started in the general region of northern Argentina and Paraguay. The 3 most primitive species occupy this area and none of the 7 most highly derived species occur in the region. From this general area, species moved in two directions. One route was up the coast of southeastern Brazil as far as the state of Parahyba, with an outpost on Ilha Trindade. The other path of migration was through Bolivia and up the Cordillera to Colombia, then eastward through Venezuela to British Guiana, with outposts in the West Indies, central Mexico, and the Galapagos Islands. These routes of migration were determined by arranging the species in a phylogenetic series and then successively comparing the ranges. Section *Eudoryopteris* has a broad distribution with relatively few, distinct, widely ranging species. It went through its species evolution rather early. Section *Lytoneuron* is not as widely distributed and has many, less distinct, local species. The species of this section evolved more recently. Its center of evolution is in or near the state of Rio de Janeiro. Of the 12 species in the section, 10 grow in the states of Rio de Janeiro and São Paulo, and 5 of them are endemic to that area.

VAN FLEET, D.S., Heidelberg College, Tiffin, Ohio. *The influence of the morphological behavior of unsaturated fat oxidases on the development and function of the endodermis*.—The unsaturated fatty acid oxidation system of the endodermis may be identified as a series of chain reactions catalyzed by metal salts and various alkaline pro-oxidants. The behavior of the system is modified by local differences in intensity of oxidation in stele and cortex so that several distinct types of morphological and functional endodermal cells appear. The Casparian strip and centripetal deposition are associated with partial and incomplete oxidation and the polymerization of degradational products. The peculiar morphological appearance of the "gap" cell is a result of oxidation without the accumulation of oxidized substances which are soluble and diffusible. The intense oxybiosis in some "gap" cells is causally related to their function as water passage cells. The starch sheath is characterized by the presence of alkali labile antioxidants which prevent oxidative decomposition of fats. These fats accumulate and inhibit diastatic action so that starch of an isotropic nature is retained. Sodium selenite, and other alkali salts, as reported previously, will inhibit oxidation in other oxidase systems but will accelerate oxida-

tion in the endodermis through the destruction of alkali labile antioxidants. By means of selenite and phosphate buffers (pH 7.2-7.8) it is possible to isolate histologically the unsaturated fat oxidase system. Thus the tissue having the morphological position of the endodermis, without any of the accepted configurations and standards for this tissue, may be identified by virtue of its functional behavior as well as by position. It is possible to demonstrate a functional and morphological endodermis in many stems reported earlier to be without this tissue.

VERDOORN, FRANS, Arnold Arboretum, Jamaica Plain, Mass. *The centenary of the "Botanische Zeitung."*—A short account of the early history of the "Botanische Zeitung," one of the first scientific and professional botanical journals, its influence, and its successors. A discussion of the task of botanical journals today, with special reference to the promotion of professional and international relations in the plant sciences.

WALLACE, RAYMOND H., University of Connecticut, Storrs, Conn. *A simple and effective automatic voltage regulator.*—A simple and effective voltage regulator suitable for many laboratory uses is described. It consists essentially of a transformer of the Variac type which is held on balance to the voltage desired by means of two small thyatron tubes that activate a reversible motor. The general characteristics of the instrument are as follows: amperage range, 5 to 50 depending upon transformer used; accuracy of control, plus or minus $\frac{1}{2}$ volt in 110; speed of correction, one to five seconds for 20 volts variation; wave form, no distortion; range of output voltage for which regulator can be set, 105 to 120, for input voltage variation from 90 to 130; accuracy with variable load, equally accurate for no load and for full load for which transformer is built; dependability, has run continuously for 14 months with only nominal attention. The cost of all materials for a 5 ampere model is about \$26.00. List of materials, instructions and full sized blueprints for the construction of the control are available.

WARMKE, H. E., Carnegie Institution, Cold Spring Harbor, N. Y. *The cytology and breeding behavior of the Russian dandelion, Taraxacum kok-saghyz.*—The chromosome number of *T. kok-saghyz* is $n = 8$; $2n = 16$, which establishes this species as a basic diploid in the genus. One pair of chromosomes bears large satellites. Plants protected from insects rarely set seed, but are fully fertile out of doors or when manually cross-pollinated, indicating a high degree of self-sterility. This species shows no evidence of apomixis, which is usual for the common polyploid members of the genus. Gamete formation follows the normal sexual pattern on both male and female sides: microsporogenesis is regular and produces a high percentage of good pollen. The macrospore mother cell undergoes a reduction division to form a linear tetrad of macrospores, the chalazal one of which usually becomes functional and forms the typical 7-celled macrogametophyte.

The above facts indicate *T. kok-saghyz* to be a normal sexual diploid and open the way for selection and breeding experiments to increase rubber content.

WEATHERWAX, PAUL, Indiana University, Bloomington, Ind. *Morphology of the spikelet of Dryzopsis hymenoides.*—The vascular bundles of the two lateral lodicules arise acropetally. The single bundle of the third (median) lodicule differentiates both acropetally and basipetally and fails to make connection with the central vascular system of the flower. This has an interesting bearing upon some recent theories as to differences between vegetative and floral organs. The point of the short, blunt callus is formed by a definite downward projection of the base of the lemma, not by an oblique abscission. This structure is much more pronounced in *Stipa* and *Aristida*. The awn breaks from the lemma by a definite absciss layer.

WETMORE, RALPH H., and ADA C. SMITH, Harvard University, Cambridge, Mass. *Development of the shoot system in Lycopodium.*—A comparative study of the stem apices in nine species of *Lycopodium* shows two major patterns for the apical meristems: (1) a flat-topped apex with erect foliar primordia; (2) a conical apex with lateral foliar primordia. In no species was a single apical initial found. In all species studied, the acropetally developing procambial column reached close to the apex, higher than the highest leaf primordium. In all species cortical tissues were slow in taking form. In the progressive developmental changes giving vascular from procambial tissues there occurred in order: (1) a centrifugal blocking out of the metaxylem and metaphloem pattern in the procambial column; (2) a differentiation of peripherally distributed, somewhat regularly spaced groups of procambial cells into alternate bundles of protoxylem and proto-phloem while stem and leaves were growing actively. At least the protoxylem strands are related to the leaf primordia. In no species investigated did phloem cells ever appear either in the leaf trace or in the leaf lamina; (3) a centripetal differentiation of metaxylem and metaphloem after active growth has slowed down. This method of development is characteristic of both superficial and subterranean rhizomes and upright axes, though differences in pattern may result. The procambial and vascular cylinders seem to be of two portions—a peripheral portion characteristically foliar, and a central cauline portion. It is significant that at times, if not always, the subterranean rhizomes of *L. obscurum* lack leaves except for fleshy scale-like structures with no veins, yet the axis has the same organization.

WHEELER, LOUIS C., Department of Botany, University of Pennsylvania, Philadelphia, Pa. *Dates of publication of Jepson's "Manual."*—W. L. Jepson's "Manual of the Flowering Plants of California" is claimed, on the back of the title-page, to have been issued as six fascicles on various dates during 1923 and 1925. Since the original fascicles are apparently unknown at least in the institutional

libraries of the U. S., verification of the dates of publication is difficult. The earliest notice that has been found which announces any part of the work for sale was published in 1926. In order to reassure ourselves that the extreme rarity of the fascicles is not evidence that they were not published on the dates claimed, we need answers to the following questions relating to and establishing the date(s) of publication of the "Manual." 1. When, where, and how was the work first announced for sale? 2. To whom were the fascicles sent, on or before the claimed dates of publication? 3. When and where was the work first reviewed or noticed? 4. What are the serial numbers of the copyrights covering the fascicles? 5. How does the text of the bound volume differ from that of the fascicles?

WILDE, MARY H., Mount Holyoke College, South Hadley, Mass. *A new interpretation of coniferous cones with special reference to the Podocarpaceae*.—Two species of *Podocarpus* possess similar compound male and female fertile branches. The female fertile branches bear spirally arranged bracts; in the axil of each is a single ovule surrounded by an epimatium (ovuliferous scale), reduced from a secondary, determinate fertile shoot and its appendages. The male fertile branches likewise bear spirally arranged bracts; in the axil of each is a small sessile male cone, a relatively unreduced secondary, determinate fertile shoot. Modifications of the male fertile branch consist in (1) internodal shortening forming primary clusters of cones, (2) loss of all cones except a single terminal one, below which are retained several persistent sterile bracts. Internodal shortening of the secondary and primary vegetative branches results in secondary and tertiary clusters respectively. Ultimately these clusters may be reduced to single cones. Reduction and loss of units have also occurred in the female fertile branches in most species. In a few other species the female fertile branch is organized into a cone. It may be concluded that primitive Coniferae possessed a richly branched habit with similar compound male and female fertile branches; that the simple male cone, a single surviving unit of the primitively compound male fertile branch, is thus homologous with the reduced, axillary secondary units of the female cone; that the great diversity in position of male cones is due to subsequent vegetative branch reduction.

WOODSON, R. E., JR., Missouri Botanical Garden, St. Louis, Mo. *Behind the scenes of a "flora of Panama"*.—Plans for a "Flora of Panama" are discussed, and certain questions raised concerning the scope and format of comprehensive floras in general, particularly with regard to the treatment of exotics. Six general categories of vascular plants are recognized in Panama upon the basis of geographical affinities: (1) Mexican-Central American, (2) Antillean, (3) northern and eastern South American, (4) Andean, (5) Panamerican, (6) Pan-tropic. Various combinations occur. The highland

flora is almost equally Mexican and Andean, but somewhat more the latter; the lowland flora is almost equally Mexican and northern South American (somewhat more of the latter), and a small but distinguishable Antillean element.

WYNNE, FRANCES E., New York Botanical Garden, New York, N. Y. *Methods of reporting plant distribution data*.—In the United States many range extensions are made and reported by amateur rather than professional botanists. Helpful suggestions to amateurs are offered herewith. (1) The title should state the contents as exactly as possible, including mention of new records, new entities, and new combinations. (2) New records should be indicated by asterisks or bold face type. (3) Information about the species concerned should be as complete as possible, including previously known range and length of time established. (4) Specimens should be deposited in as many prominent herbaria as possible, and mention should be made of these. (5) Maps showing distribution are often of value.

ZIMMERMAN, P. W., and HITCHCOCK, A. E., Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y. *Correlation of organs and flowering habit modified by triiodobenzoic acid*.—Triiodobenzoic acid has a formative influence on plants, affecting flowering, growth habits, and correlation of organs. Solutions of the substance were applied to the soil or sprayed on the plants with similar results. One to 10 mg. added to the soil of a four-inch pot were sufficient. As a spray 25 to 500 mg./l. served for the various responses. Under the influence of this hormone-like substance, axillary buds which normally produce leafy shoots were induced to grow flower clusters. The main shoot of the plant also lost the shoot-producing bud and terminated in flower clusters. It was concluded that though triiodobenzoic acid does not cause immediate (within a few hours) cell elongation of test objects, the ultimate results more nearly resemble those of true hormones than the well known indole and naphthalene substances commonly referred to as auxins. The term "formagen" is proposed for substances which have a formative effect on plants involving modification of shape, size, and pattern of organs and flowering habit.

ZIMMERMAN, P. W., and A. E. HITCHCOCK, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y. *Peculiarities in physiological activity of homologs of substituted phenoxyacetic acids*.—Substituted phenoxy compounds have varying degrees of physiological activity as plant growth substances according to the kind (halogen, amino, nitro, or methyl), number, and location on the ring of substituted groups. The activity is further conditioned by the length of side chain and place of attachment to the oxygen. Activity depends upon molecular configuration as a whole rather than upon any one part of the molecule. Substituted phenoxy substances resemble the well known auxins in that they cause cell elongation, epinasty of leaves, and induce adventitious roots; they differ in that they

have a special formative influence on growth organs. The latter effects are exhibited by modification of form and color of leaves, flowers, and fruit. Illustrating peculiarities, 2,4-dichlorophenoxyacetic acid causes both cell elongation and modification of leaves; 2,4-dichlorophenoxy-alpha-propionic acid induces cell elongation but does not modify leaves,

while the beta form is inactive for both responses; 2,4-dichlorophenoxy-alpha-butyric acid is active for cell elongation but does not modify leaves; 2,4-dichlorophenoxymalonic acid is active for both cell elongation and modification of leaves. Other substitutions and homologs show similar as well as other peculiarities.